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Provisional Peer Reviewed Toxicity Values for

Cobalt
(CASRN 7440-48-4)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
μg	microgram
μmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR COBALT (CASRN 7440-48-4)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   < Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   < California Environmental Protection Agency (CalEPA) values, and
   < EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

The Integrated Risk Information System (IRIS) does not report a Reference Dose (RfD) for cobalt (U.S. EPA, 2007). The Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997a) and Drinking Water Standards and Health Advisories list (U.S. EPA, 2004) likewise do not contain an RfD for cobalt. The Chemical Assessments and Related Activities (CARA) lists (U.S. EPA, 1991, 1994a) report a Health Effect Assessment (HEA) for cobalt (U.S. EPA, 1987). The 1987 HEA derived a chronic RfD of 0.005 mg cobalt/kg-day based on a no-observed-adverse-effect level (NOAEL) of 5 mg cobalt/kg-day for testicular effects in a subchronic rat study (Nation et al., 1983). The Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for cobalt and its compounds reports an oral Minimal Risk Level (MRL) for intermediate exposure of 1×10^{-2} mg/kg-day (ATSDR, 2004), based on a lowest-observed-adverse-effect level (LOAEL) of approximately 1 mg cobalt/kg-day for polycythemia in humans (Davis and Fields, 1958). ATSDR (2004) did not derive an oral MRL for chronic exposure. This MRL for intermediate exposure was based on the polycythemic effect of cobalt exposure (1 mg cobalt/kg-day, Davis and Fields, 1958) by application of an UF

of 10 for a LOAEL and an UF of 10 for human variability. The World Health Organization (WHO, 2005) has not published an Environmental Health Criteria (EHC) document about cobalt. An International Agency for Research on Cancer (IARC) Monograph on cobalt and its compounds (IARC, 1991) and the National Toxicology Program (NTP) Status Reports (NTP, 2005) were searched for relevant information.

IRIS (U.S. EPA, 2007) does not report a Reference Concentration (RfC) for cobalt. The HEAST (U.S. EPA, 1997a) likewise does not list an RfC for cobalt. The cobalt HEA (U.S. EPA, 1987) derived a subchronic inhalation RfC of $9x10^{-5}$ mg/m^3 based on a LOAEL of 0.1 mg/m^3 for respiratory effects in a 3-month study in swine (Kerfoot et al., 1975). A chronic inhalation RfC of $9x10^{-6}$ mg/m^3 was derived from the same study. The ATSDR Toxicological Profile for cobalt and its compounds reports an inhalation MRL for chronic exposure of $1x10^{-4}$ mg/m^3 (ATSDR, 2004), based on a NOAEL of 0.0053 mg cobalt/m^3 for decreased pulmonary function in humans (Nemery et al., 1992). The American Conference of Governmental Industrial Hygienists (ACGIH, 2004) has set a Threshold Limit Value-Time-Weighted Average (TLV-TWA) of 0.02 mg/m^3 for cobalt and inorganic cobalt compounds, expressed as cobalt, based on respiratory and cardiovascular effects. The National Institute for Occupational Safety and Health (NIOSH, 2005) Recommended Exposure Limit (REL) TWA for cobalt is 0.05 mg/m^3, based on effects in the respiratory system. The Occupational Safety and Health Administration (OSHA, 2005) Permissible Exposure Limit (PEL) is 0.1 mg/m^3.

IRIS (U.S. EPA, 2007) does not report a cancer classification, slope factor or unit risk for cobalt. The HEAST (U.S. EPA, 1997a) and Drinking Water Standards and Health Advisories list (U.S. EPA, 2004) likewise do not report carcinogenicity assessments for cobalt. The CARA lists (U.S. EPA, 1991, 1994a) do not report a cancer classification or an estimate of the carcinogenic potency of stable cobalt compounds due to a lack of pertinent data. An IARC Monograph on cobalt and its compounds (IARC, 1991) classified cobalt and its compounds as "possibly carcinogenic to humans." ACGIH (2004) has classified cobalt in category A3 – confirmed animal carcinogen with unknown relevance to humans.

Literature searches for studies relevant to the derivation of provisional toxicity values for cobalt were conducted from 1991 to 2000 in TOXLINE (supplemented with BIOSIS and NTIS updates), MEDLINE, TSCATS, RTECS, CCRIS, DART, EMIC/EMICBACK, HSDB, GENETOX and CANCERLIT and from 2000 to August 2005 in MEDLINE, TOXLINE (NTIS subfile), TOXCENTER, TSCATS, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS and Current Contents.

REVIEW OF PERTINENT DATA

Human Studies

Overview

Indicators of adverse health effects in humans following oral exposure to cobalt include increased erythrocyte number and hemoglobin (Taylor et al., 1977; Duckham and Lee, 1976; Davis and Fields, 1958), cardiomyopathy (Morin et al., 1971; Alexander, 1969, 1972) and decreased iodine uptake by the thyroid (Roche and Layrisse, 1956). Cardiomyopathy is an endpoint of concern for cobalt in humans; however, it is highly likely that alcohol consumed in "beer-cobalt cardiomyopathy," as well as other factors, such as smoking, played a role in the effects that were observed. Cobalt is a sensitizer in humans by any route of exposure. Sensitized individuals may react to inhalation of cobalt by developing asthma; ingestion or dermal contact with cobalt may result in development of dermatitis. Several studies have suggested that cross-sensitization may occur between cobalt and nickel (Shirakawa et al., 1990; Lammintausta et al., 1985; Bencko et al., 1983; Rystedt and Fisher, 1983).

Respiratory effects, including respiratory irritation, wheezing, asthma, pneumonia and fibrosis, have been widely reported in humans exposed to cobalt by inhalation (ATSDR, 2004). Epidemiology studies show decreased pulmonary function in workers exposed to inhaled cobalt (Nemery et al., 1992; Gennart and Lauwerys, 1990). Results of studies investigating cancer incidence in workers exposed to inhaled cobalt are suggestive of a possible association between exposure to cobalt and respiratory tumors (Tuchsen et al., 1996; Mur et al., 1987; Morgan, 1983).

Oral Exposure

In humans, cobalt stimulates production of red blood cells through increased production of the hormone erythropoietin and has been explored for use in the treatment of anemia (Smith and Fisher, 1973; Duckham and Lee, 1976). Increases in red blood cell counts and blood hemoglobin have been reported in non-anemic volunteers (Davis and Fields, 1958) and in anephric anemic patients (Taylor et al., 1977; Duckham and Lee, 1976).

Reversible polycythemia (increase in blood cell number) was reported (Table 1) in six healthy adult males following treatment with 150 mg cobalt chloride per day for 22 days (Davis and Fields, 1958). Five subjects received 150 mg cobalt chloride/day for the entire exposure period and a sixth subject initially received 120 mg cobalt chloride/day, which was later increased (time not specified) to 150 mg/day. Cobalt chloride was administered as a 2% solution diluted in either water or milk. Assuming an average body weight of 70 kg, 150 mg cobalt chloride/day corresponds to approximately 1 mg cobalt/kg-day. Outcomes assessed in this study were red blood cell count, hemoglobin percentage, leukocyte count, reticulocyte percentage and thrombocyte count. Polycythemia was observed in all six patients within 7 to 22 days of

Table 1. Hematopoietic, Thyroid and Developmental Effects of Cobalt via Oral Route

Target Organ	Species	Effect	Dosage (mg/kg-day)
Hematopoietic Effects	Human	Reversible Effect (Polycythemia)	1.0
	Human	↑ Hemoglobin and ↑ RBC	0.04 – 0.14*
	Rat	Hematopoietic effect	0.5 – 40.0
Thyroid	Human	↓ Iodine uptake	1.0
	Mice	Histopathological changes in thyroid	26.0
Fetus	Rat	Developmental toxicity	5.2 – 21.0
Heart	Rat	↓ Myocardial function	8.0

*Therapeutic dose for anemic patients

treatment as demonstrated by increases in red blood cell counts ranging from 0.5 to 1.19 million (approximately 16-20% increase above pre-treatment levels) and increases in hemoglobin levels ranging from 6 to 11% above pretreatment values. In five of the six subjects, reticulocyte levels were elevated, reaching at least twice the pre-experiment values. Thrombocyte and total leukocyte counts were not significantly different from pretreatment values. Erythrocyte counts returned to pre-treatment levels within 9 to 15 days after cobalt administration was discontinued. The fact that leucocyte counts remained relatively constant throughout the experiment supports the concept that this is a true polycythemia.

Duckham and Lee (1976) treated 12 anephric patients on dialysis with 25 to 50 mg cobalt chloride daily for approximately 12 weeks. Assuming an average body weight of 70 kg, doses of 25 and 50 mg cobalt chloride/day are equivalent to 0.16 and 0.32 mg cobalt/kg-day, respectively. During the exposure period, patients also received daily treatment with 100 mg ferrous sulfate and 50 mg ascorbic acid. Within approximately 2 months of initiation of treatment with cobalt, an increase in hemoglobin of 26-70% was observed in patients treated with 50 mg cobalt chloride/day. Serum cobalt levels appeared to reach steady state within 2 months of exposure (approximately 40-100 µg cobalt/100 mL). In a subgroup of three patients, continuation of treatment with 25 mg cobalt chloride/day for approximately 3 months maintained elevated hemoglobin levels. Hemoglobin levels decreased rapidly when cobalt therapy was discontinued. The authors did not report whether therapy with ferrous sulfate and ascorbic acid was discontinued at the same time. Results of this study are difficult to interpret because patients were anephric and on dialysis, which may have altered cobalt pharmacokinetics and dose-effect relationships. Furthermore, since it is well established that treatment with ferrous sulfate alone increases hemoglobin concentration (Hillman, 2001), concomitant therapy with iron is a confounding factor. Since this study did not evaluate the response of patients treated with ferrous sulfate alone, it is not possible to determine the relative contributions of iron and cobalt to the observed increases in hemoglobin. A group of eight anephric patients with refractory anemia were treated with 25 to 50 mg cobalt chloride daily for 12 to 36 weeks (Taylor et al., 1977). Increased hemoglobin concentration and decreased requirement for blood transfusions were observed (Taylor et al., 1977). Data on hemoglobin concentrations (or other indicators of polycythemia) were not reported.

Pregnant women given 75 to 100 mg cobalt chloride/day with no other treatment for 90 days to 6 months did not experience pregnancy-induced reductions in hematocrit and hemoglobin levels, compared to untreated controls (Holly, 1955). However, daily treatment with 1 g ferrous sulfate alone or combined daily treatment with 60 to 90 mg cobalt chloride and 0.8 to 1.2 g ferrous sulfate prevented pregnancy-related decreases in hematocrit and hemoglobin levels. The response to combined cobalt chloride and iron therapy was more pronounced than the response to iron therapy alone. In patients treated with iron only, decreases in hemoglobin and hematocrit were prevented in approximately 80% of patients, compared to 100% of patients treated with combined cobalt chloride and iron.

Cardiomyopathy has been observed in association with consumption of large quantities of beer containing cobalt chloride (introduced into the beer to stabilize the foam) (Alexander, 1969, 1972; Morin et al., 1971). Exposure estimates in reported cases range from 0.04 to 0.14 mg cobalt/kg-day (corresponding to approximately 8-30 pints of beer daily) over a period of years (Alexander, 1969, 1972; Morin et al., 1971). The cardiomyopathy in the beer drinkers, referred to in the literature as "beer-cobalt cardiomyopathy," was fatal to 43% of the subjects within several years, with approximately 18% of these deaths occurring within the first several days following diagnosis. Beer-cobalt cardiomyopathy appeared to be similar to alcoholic cardiomyopathy and beriberi; however, the onset of the beer-cobalt cardiomyopathy was much more abrupt. The practice of adding cobalt to beer to stabilize the foam has been discontinued. It should be noted, however, that the cardiomyopathy may also have been due to the fact that the beer drinkers had protein-poor diets and may have had prior or concurrent cardiac and hepatic damage from alcohol abuse. Cobalt exposure levels in the anemia studies were similar to or higher than those estimated for the cardiomyopathy studies (0.04 to 0.14 mg cobalt/kg-day); however, exposure duration in the anemia studies was much shorter, ranging from 22 days to 36 weeks, compared to an estimated exposure period of years for the cardiomyopathy studies.

The thyroid also appears to be a target organ for cobalt (Table 1). Treatment of 12 euthyroid (normal thyroid) patients with 150 mg cobalt chloride/day (equivalent to 1 mg cobalt/kg-day, assuming a body weight of 70 kg) for 2 weeks resulted in a greatly reduced uptake of 48-hour radioactive iodine by the thyroid when measured after 1 week of exposure to cobalt, with uptake nearly abolished completely by the second week of exposure to cobalt (Roche and Layrisse, 1956). When cobalt treatment was discontinued, radioiodine uptake returned to pre-treatment reported values. In a small clinical study, decreased radioactive iodine uptake was reported in two of four euthyroid patients administered 37.5 mg cobalt /day as cobalt chloride (equivalent to 0.54 mg cobalt/kg-day, assuming a body weight of 70 kg) for 10 to 14 days. While reviewing this report, it was discovered that one of the two subjects with reported decreased iodine uptake has received i.v. cobalt in addition to oral cobalt intake. The i.v. dosing may have raised the internal concentration to a level greater than the reported 0.54 mg dosage based upon oral dosing of 37.5 mg/day in other subjects that did not receive i.v. dosing. Lack of details pertinent to other clinical conditions (including effects on thyroid stimulating hormone [TSH]) of these patients are not available; thus the mechanism for the effect of cobalt on thyroidal iodine uptake cannot be ascertained. Cobalt appears to increase thiocyanate-induced release of radioiodine from the thyroid, suggesting a possible effect on binding of iodine (e.g.,

iodination of thyroglobulin) in the thyroid gland (Paley et al., 1958).Furthermore, the changes in reduced iodine uptake whether operate through same mechanism as the changes in erythrocyte numbers has not been determined (ATSDR, 2004).

Occupational exposure to semi-soluble cobalt had significant effect on thyroid hormones which was correlated with increased levels of urinary cobalt (Prescott et al., 1992). However, serum thyroid simulating hormones remained unaltered following long-term cobalt exposure. As reported by these authors, the mechanism of cobalt on thyroid hormones cannot be explained. Furthermore, as reported in the report of Prescott et al. (1992), long-term oral doses of cobalt (2-4 mg/kg-day) in anemic children has been reported to produce goiter. The goitrogenic dose (2-4 mg/kg-day) effect in anemic children is reportedly observed at a much higher dosage than the dosage (0.54 mg/kg-day) reported in Paley et al. (1958). No other additional data are available to elucidate mechanism of cobalt on thyroid metabolism or iodine uptake (ATSDR, 2004).

Cobalt has been found to be a sensitizer in humans. Individuals are sensitized following dermal or inhalation exposure, but flares of dermatitis may be triggered following cobalt ingestion. In a small clinical study, several patients with eczema of the hands were challenged orally with 1 mg cobalt (0.014 mg cobalt/kg-day as cobalt sulfate) in tablet form once per week for 3 weeks; 28/47 patients had a flare of dermatitis following the oral challenge (Veien et al., 1987). All 47 patients had positive dermal patch tests to cobalt (13 to cobalt alone and 34 to nickel and cobalt) and 7 of the 13 patients who had patch-tested positive to cobalt alone reacted to the oral challenge. These results suggest that cobalt allergy can be induced from ingestion exposures to cobalt. The exposure levels associated with sensitization to cobalt following inhalation or dermal exposure have not been established.

Interrelationships have been found to exist between cobalt and nickel sensitization (Bencko et al., 1983; Rystedt and Fisher, 1983; Veien et al., 1987). In guinea pigs, nickel and cobalt sensitization appear to be interrelated and mutually enhancing (Lammintausta et al., 1985). Therefore, it is possible that in people sensitized by nickel, exposure to cobalt may result in an allergic reaction.

Inhalation Exposure

Numerous studies have investigated health effects in workers occupationally exposed to cobalt-bearing dust (Linna et al., 2003; Swennen et al., 1993; Auchincloss et al., 1992; Cugell, 1992; Nemery et al., 1992; Prescott et al., 1992; Gennart and Lauwerys, 1990; Meyer-Bisch et al., 1989; Raffn et al., 1988; Shirakawa et al., 1988, 1989; Sprince et al., 1988; Kusaka et al., 1986a,b; Demedts et al., 1984; Davison et al., 1983). However, many of these studies are of limited utility for risk assessment due to inadequate characterization of exposure and/or effects. Four studies were considered to be potentially suitable bases for RfC derivation. Two of these focused exclusively on respiratory effects (Nemery et al., 1992; Gennart and Lauwerys, 1990); one studied only thyroid effects (Prescott et al., 1992) and one considered multiple endpoints (Swennen et al., 1993). The populations studied included diamond-cobalt saw manufacturers,

diamond polishers, plate painters and cobalt production workers. All four studies were cross-sectional design.

Several studies have examined the effects of hard metal, a mixture containing approximately 20% cobalt with the remainder being primarily tungsten carbide. Exposure of humans to hard metal has been shown to result in an increase in cancer mortality (Moulin et al., 1998; Lasfargues et al., 1994) as well as a number of other diseases, including asthma and fibrosis (for reviews, see Barceloux, 1999; Lison, 1996). There is substantial evidence from animal studies that tungsten, although it acts as an inert dust by itself, can potentiate the effects of cobalt on the respiratory tract (Lasfargues et al., 1995; Lison et al., 1995, 1996; Swennen et al., 1993). For this reason, studies of hard metal were not given further consideration.

Gennart and Lauwerys (1990) studied ventilatory function in workers at a plant producing diamond-cobalt circular saws. The form of cobalt used in diamond polishing is primarily metallic cobalt. The exposed population consisted of 48 workers (34 males and 14 females) who agreed to participate in the study (an additional 27 workers declined). Exposure duration for these workers ranged from 0.1 to 32 years, with an average of approximately 6 years. The work involved weighing and mixing cobalt powder and microdiamond particles (and possibly small amounts of other undisclosed substances), cold pressing, heating and hot pressing. After sintering, the pieces were welded onto steel disks. These operations were performed in two rooms called the mixing room and the oven room, where all the examined workers spent most of their time. Controls were 23 workers (11 males and 12 females) from other factories in the same area who were not exposed to known pneumotoxic chemicals. Personal air samples were collected at different workplaces during half a workshift. Subjects filled out a questionnaire regarding occupational and medical histories, smoking habits and pulmonary symptoms; gave a urine sample for cobalt determination; and submitted to lung function tests. Cobalt concentrations varied from 9.4 to 2875 $\mu g/m^3$ in the mixing room (geometric mean=135.5 $\mu g/m^3$) and from 6.2 to 51.2 $\mu g/m^3$ in the oven room (geometric mean=15.2 $\mu g/m^3$). The prevalence of respiratory symptoms, such as cough, sputum and dyspnea, were significantly increased in the exposed workers compared to the control group (numeric data not reported). Mean predicted values of FEV_1 (forced expiratory volume in 1 second adjusted for body size) and FVC (forced vital capacity) were significantly lower, and the prevalence of abnormal values was higher in the exposed workers (both smokers and non-smokers) compared to the control group. In controls, FEV_1 and FVC were 95.4 and 101.6 percent of predicted values, respectively. Mean percent predicted FEV_1 and FVC in exposed non-smokers were 87.1 and 92.3, respectively, and in exposed smokers were 83.9 and 93.4, respectively. Among non-smokers, all measures of pulmonary function were lower in workers exposed for 5 years or more than in those exposed for a shorter period of time.

Nemery et al. (1992) conducted a cross-sectional study of cobalt exposure and respiratory effects in diamond polishers. The study group was composed of 194 polishers working in 10 different workshops. In two of these workshops (#1, 2), the workers used cast iron polishing disks almost exclusively, and in the others, they primarily used cobalt-containing disks. The number of subjects from each workshop varied from 6 to 28 and the participation rate varied

from 56 to 100%. The low participation in some workshops reflects the fact that only workers who used cobalt disks were initially asked to be in the study; low participation is not due to a high refusal rate (only eight refusals were documented). More than a year after the polishing workshops were studied, an additional three workshops with workers engaged in sawing diamonds, cleaving diamonds or drawing jewelry were studied as an unexposed control group (n=59 workers). Subjects were asked to fill out a questionnaire regarding employment history, working conditions, medical history, respiratory symptoms and smoking habits; to give a urine sample for cobalt determination; and to undergo a clinical examination and lung function tests. Both area air samples and personal air samples were collected (always on a Thursday). Sampling for area air determinations started 2 hours after work began and continued until 1 hour before the end of the work day. Personal air samples were collected from the breathing zone of a few workers per workshop for four successive 1-hour periods. Air samples were analyzed for cobalt and iron. In addition, personal air samplers were used to sample the air 1 cm above the polishing disks. These samples were analyzed for the entire spectrum of mineral and metallic compounds. Air samples were not obtained at one of the polishing workshops (#4); however, this workshop was reported to be almost identical to an adjoining workshop (#3) for which samples were obtained. Urinary cobalt levels were similar between workers in these two workshops, so exposure was considered to be similar as well.

Results of area and personal air sampling were strongly correlated (R=0.92), with area air sampling reporting lower concentrations than personal air samples in all workshops except one (#9) (Nemery et al., 1992). In this workshop, personal air samples appeared to be artificially low in comparison to area air samples and urinary cobalt levels of the workers. When this workshop was excluded, a strong correlation (R=0.85-0.88) between urinary cobalt and cobalt in the air was observed. Based on urinary cobalt levels, the predicted concentration of cobalt expected in personal air samples from workshop #9 was approximately 45 $\mu g/m^3$ (the mean value actually reported was 6 $\mu g/m^3$). The polishing workshops were divided into two groups: those with low exposure to cobalt (#1-5, n=102) and those with high exposure to cobalt (#6-10, n=91). Mean cobalt exposure concentrations were 0.4, 1.6 and 10.2 $\mu g/m^3$ by area air sampling and 0.4, 5.3 and 15.1 $\mu g/m^3$ by personal air sampling in the control, low-exposure and high-exposure groups, respectively. The inclusion of the apparently biased personal air samples from workshop #9 means that the reported mean cobalt exposure in the high-exposure group obtained by personal air sampling (15.1 $\mu g/m^3$) may be lower than the true value. Air concentrations of iron were highest in the two polishing workshops that used iron disks and the sawing workshop (highest value=62 $\mu g/m^3$), and were not correlated with cobalt levels. Analysis of samples taken near the disks showed the presence of cobalt, with occasional traces of copper, zinc, titanium, manganese, chromium, silicates and silicon dioxide. No tungsten was detected. Some workers may have previously been exposed to asbestos since pastes containing asbestos had been used in the past to glue the diamonds onto holders. However, since the asbestos was in its non-friable form exposure was insufficient to produce functional impairment. Smoking habits were similar in workers from the high-exposure, low-exposure and control groups. Duration of exposure was not discussed.

Workers in the high-exposure group were more likely than those in the other groups to complain about respiratory symptoms; the prevalences of eye, nose and throat irritation and cough, and the fraction of these symptoms related to work, were significantly increased in the high-exposure group (Nemery et al., 1992). Workers in the high-exposure group also had significantly lower lung function compared to controls and low-exposure group workers, as assessed by FVC, FEV_1, MMEF (forced expiratory flow between 25 and 75% of the FVC) and mean PEF (peak expiratory flow rate), although the prevalence of abnormal values did not differ significantly between exposure categories. In controls, FVC, FEV_1 and MMEF were approximately 110, 107 and 94 percent of predicted values, respectively, compared to approximately 105, 104 and 87 percent of predicted values, respectively, in the high-exposure group workers. Results in the low-exposure group did not differ from controls. The effect on spirometric parameters in the high exposure group was present in both men and women. Women seemed to be affected more than men; however, the interaction between exposure and sex was not significant (two-way analysis of variance). Smoking was found to exert a strong effect on lung function; however, lung function level remained negatively correlated with exposure to cobalt, independent of smoking.

A cobalt dose-effect relationship is evident from the Nemery et al. (1992) study, based on a multivariate regression analysis of urinary cobalt and lung function measurements. Increasing urinary cobalt concentration (approximate range <1-70 µg cobalt/g creatinine) was significantly ($p<0.05$) associated with co-variate-adjusted decreasing forced expiratory volume (FEV1%) and forced vital capacity (FVC%). Significant co-variates retained in the regression analysis included gender and smoking. The model predicted 3% and 4% decreases in FEV1% and FVC%, respectively, in association with a 10-fold increase in urinary cobalt concentration. The approximate mean urinary cobalt levels of the control and high exposure groups were 2 and 20 :g cobalt/g creatinine, respectively. The magnitude of the cobalt effect was similar to the predicted effect of smoking, approximately 3-4% decrease in FEV1% and FVC%.

Swennen et al. (1993) conducted a cross-sectional study of workers exposed to metallic cobalt and inorganic cobalt compounds at a cobalt plant producing these materials from cobalt metal cathodes and scrap metal. The study group included 82 male workers from the cobalt plant who had no history of lung disease prior to employment and who had never been exposed to other pneumotoxic chemicals. Methods for selection or exclusion of subjects in constructing the cohort and participation were not reported. The control group comprised 82 age-matched workers from the mechanical workshop of a nearby plant owned by the same company. Workers filled out a questionnaire regarding occupational history, respiratory complaints and smoking habits; received a routine clinical examination; submitted to lung function tests; had a chest radiograph taken; and gave blood and urine samples (before and after working on Monday and Friday of one week) for determination of cobalt content as well as hematological and serum chemistry analyses. Exposure was monitored by personal air samplers worn by each cobalt worker for 6 hours on both Monday and Friday.

Workers in the cobalt plant were exposed to cobalt concentrations ranging from 1 to 7772 $\mu g/m^3$ (Swennen et al., 1993). The geometric mean exposure concentration was 125 $\mu g/m^3$.

Exposure duration ranged from 0.3 to 39.4 years, with an average exposure of 8.0 years. A significantly higher number of exposed workers reported dyspnea than did controls. The increase occurred primarily among smokers although no significant interaction was found between smoking and exposure to cobalt. Based on a logistic regression model, the probability of dyspnea during exercise was significantly associated with increasing cobalt concentration in the air or urine. The parameters of the model were not reported. The clinical examinations detected significantly increased prevalence of skin disorders (eczema, erythema) (51 vs. 25%) and wheezing (16 vs. 6%) in the exposed group compared to controls. Lung function tests did not differ between the two groups; however, a few significant trends were noted: the FEV_1/VC (forced expiratory volume in one second/vital capacity) ratio decreased with increasing concentration of cobalt in the air and urine, and the RV (residual volume) and TLC (total lung capacity) increased with increasing duration of exposure. No lung abnormalities were found by chest radiographs in either group. Blood analyses did not show polycythemia, and in fact, there were slight, but significant, decreases in red blood cell count, hemoglobin and hematocrit in the exposed workers. White blood cell counts were significantly increased. Serum levels of the thyroid hormone T3 (triiodothyronine) were slightly (7%), but significantly, decreased in the exposed group, while T4 (thyroxine) and TSH (thyrotropin) were not affected. Serum markers for cardiomyopathy (i.e., myocardial creatine kinase) were unchanged.

Prescott et al. (1992) conducted a cross-sectional study to investigate the effects of cobalt exposure on thyroid volume in female plate painters. The test group included 61 female plate painters exposed to cobalt blue dyes in two porcelain factories. The control group consisted of 48 unexposed women working at the same factories. The dyes used in the two factories differed; factory I (36 workers) used cobalt aluminate, which is insoluble, and factory II (25 workers) used cobalt-zinc silicate, which was reported to be "semi-soluble." Workers were exposed to cobalt during the painting procedure when the plates were spray-painted (under a fume hood) two or three times with the water-based cobalt blue underglaze and when the excess color was removed with a brush after drying. Cobalt concentrations were reported to be approximately 0.05 mg/m^3 in the workplaces (no further details on air levels were reported). The average duration of exposure was 14.6 years in group I workers and 16.2 years in group II workers. Subjects filled out a questionnaire regarding health, use of medicines, day of menstrual cycle, employment information and smoking habits and agreed to give blood and urine samples for determination of thyroid hormones and cobalt, respectively, and to undergo ultrasonography to determine volume of the thyroid gland.

Urinary cobalt levels were similar in group I exposed workers and controls (Prescott et al., 1992). Group II workers had urinary cobalt levels that were approximately 10-fold higher than controls. Group I workers did not differ from controls for any of the thyroid parameters measured; however, Group II workers had a significant 22% increase in serum T4 (thyroxine) levels. Mean thyroid volume was lower in this group as well although the difference from controls (16.1 mL in group II vs. 19.2 mL in controls and 18.7 mL in group I) was not statistically significant. The occurrence of respiratory effects in these workers was not reported.

Results of three studies investigating cancer incidence in workers exposed to cobalt (Tuchsen et al., 1996; Mur et al., 1987; Morgan, 1983) are suggestive of a possible association between exposure to cobalt and respiratory tumors. Morgan (1983) investigated the health and causes of death of 49 men occupationally exposed to cobalt salts and oxides in a manufacturing plant in South Wales. During the study period, 33 men died (five with lung cancer and three with cancer at other sites). The expected number of deaths was 3.0 for lung cancer and 4.1 for cancers at other sites, based on national statistics, resulting in mortality ratios of 1.7 and 0.73, respectively (statistical analysis of data not reported). U.S. EPA (1987) concluded that the scope of this study was too limited to demonstrate the carcinogenicity or noncarcinogenicity of occupational exposure to cobalt compounds.

Mur et al. (1987) analyzed the mortality of a cohort of 1143 workers in a plant that refined and processed cobalt and sodium. An increase in deaths [Standard Mortality Ratio (SMR) = 4.66; 95% confidence interval (CI) = 1.46-10.64] resulting from lung cancer was observed in workers based on four cases observed in the exposed group and one case expected based on French national statistics. In a study within the cohort that controlled for age and smoking habits, 44% (four workers) in the exposed group and 17% (three workers) in the control group died of lung cancer. The authors indicated that the differences were not statistically significant and that the workers were exposed to arsenic and nickel in addition to cobalt. The exposure levels of cobalt were not reported.

Tuchsen et al. (1996) analyzed the cancer incidence of a cohort of 874 women who worked in one of two factories (382 from one factory, 492 from a second factory) applying a cobalt-based (cobalt-aluminate spinel) plate underglaze. From unexposed areas of factory I 520 referents were selected. Both groups were compared to statistics for all Danish women in the same calendar year. During the 5-year follow-up period, the overall cancer incidence was only slightly elevated in exposed workers, while the incidence of lung cancers was significantly increased [Standard Incidence Ratio (SIR) = 2.35; 95% CI = 1.01-4.6]. The incidence of lung cancers in the referents (not exposed to cobalt) was greater than that of all Danish women, but the difference was not statistically significant. Exposure characterization prior to 1980 was not described, while exposures after 1980 were variable and reported as a mean concentration for a given year. Exposures were generally in the range of 0-1 mg cobalt/m^3 except for 2 years, during which they were greater.

Animal Studies

Overview

Studies in animals show that oral exposure to cobalt produces effects similar to those observed in humans, including increases in red blood cells and hemoglobin (Domingo et al., 1984; Krasovskii and Fridlyand, 1971; Murdock, 1959; Holly, 1955; Stanley et al., 1947), thyroid effects (Shrivastava et al., 1996) and cardiac effects (Haga et al., 1996; Pehrsson et al., 1991; Mohiuddin et al., 1970). Other findings in animals not reported in humans include

neurobehavioral changes (Singh and Junnarkar, 1991; Bourg et al., 1985; Krasovskii and Fridlyand, 1971) and testicular toxicity (Anderson et al., 1992, 1993; Pedigo et al., 1988; Corrier et al., 1985; Mollenhauer et al., 1985; Domingo et al., 1984; Nation et al., 1983). Developmental toxicity studies in rats and mice provide evidence that high oral doses of cobalt may produce developmental effects in animals, in some cases in the absence of overt maternal toxicity (Szakmary et al., 2001; Paternain et al., 1988; Domingo et al., 1985).

Animal data support the conclusion that the respiratory tract is the critical target for inhaled cobalt (NTP, 1991; Bucher et al., 1990; Wehner et al., 1977). Subchronic inhalation exposure to cobalt resulted in cytotoxicity and reparative proliferation in all regions of the respiratory tract in rats and mice (NTP, 1991; Bucher et al., 1990). Available chronic animal studies have demonstrated the carcinogenic potential of inhaled cobalt in male and female rats and mice, with alveolar and bronchiolar tumors being the most prevalent (Bucher et al., 1999; NTP, 1998).

Oral Exposure

Studies in rats show that subchronic oral exposure to cobalt chloride increases red blood cells and hemoglobin with NOAELS ranging from 0.05 to 0.62 mg cobalt/kg-day (Krasovskii and Fridlyand, 1971; Stanley et al., 1947) and LOAELs ranging from 0.5 to 30 mg cobalt/kg/day (Domingo et al., 1984; Krasovskii and Fridlyand, 1971; Murdock, 1959; Holly, 1955; Stanley et al., 1947). In general, effects in animal studies were observed at higher exposure levels than those reported in humans.

Effects of cobalt on red blood cells and hemoglobin were investigated in Sprague-Dawley rats treated with 2.5, 10, and 40 mg cobalt chloride hexahydrate/kg-day (equivalent to 0.62, 2.5, and 9.9 mg Co/kg-day, respectively) for 8 weeks (Stanley et al., 1947). After 8 weeks of exposure, increases in hemoglobin and red blood cell number were observed in the 10 and 40 mg cobalt chloride hexahydrate/kg-day treatment groups. Statistical significance was not reported.

Hemoglobin and hematocrit were significantly increased in rats exposed to 500 ppm cobalt chloride in drinking water, equivalent to approximately 30 mg cobalt/kg-day, for 3 months (Domingo et al., 1984). Compared to controls, hematocrit and hemoglobin were both increased by approximately 30% at the end of the 3-month exposure period, with increases observed within the first 2 weeks of exposure (numeric data not presented). Following the 3-month exposure period, histopathological examination showed no treatment-related morphological or ultrastructural changes to any organ. Increased tissue weights were observed for spleen, heart and lungs, and testicular weight was decreased compared to controls.

In rats exposed to 40 mg cobalt chloride/kg-day (equivalent to 18 mg cobalt/kg-day) for 4 months, hemoglobin and red blood cell count were increased by 37 and 21%, respectively, compared to controls (Holly, 1955). Similar effects were observed following concomitant administration of 40 mg cobalt chloride/kg-day and 200 mg ferrous sulfate, with increases of

30% for hemoglobin and 32% for red blood cell count, compared to controls. Statistical significance was not reported.

Oral exposure of rats to 10 mg cobalt/kg-day for 5 months resulted in increases in hemoglobin, hematocrit and red blood cell count compared to untreated controls, with effects reaching a plateau after approximately 60 days of exposure (Murdock, 1959). Statistical significance was not reported. No changes were observed for mean corpuscular hemoglobin concentration and mean cell volume compared to untreated controls, indicating that stimulation of erythropoiesis by cobalt did not result in the production of abnormal red blood cells.

The effects of exposure to 0.05, 0.5, and 2.5 mg cobalt/kg-day for 7 months were examined in rats (Krasovskii and Fridlyand, 1971). Treatment with 0.5 and 2.5 mg cobalt/kg-day, but not 0.05 mg cobalt/kg-day, for 7 months increased red blood cells and hemoglobin. Stimulation of hematopoiesis was more pronounced in the 2.5 mg cobalt/kg-day group than in the 0.5 mg cobalt/kg-day group, with polycythemia in the 0.5 mg cobalt/kg-day group described as mild and transient. Results of this study are difficult to evaluate since numeric data and statistical analyses were not reported.

Studies in animals have noted cardiac effects following cobalt (cobalt sulfate) exposure (Haga et al., 1996; Pehrsson et al., 1991; Mohiuddin et al., 1970) although at higher exposure levels than observed in human studies. The effect of cobalt on myocardial function was examined in rats exposed to 8.4 mg cobalt/kg-day for 16 or 24 weeks (Haga et al. 1996). After 24 weeks of exposure, decreased left ventricular systolic and diastolic function was observed. An increase in the ventricular weight to body weight ratio indicates that left ventricular hypertrophy is a contributory factor in cobalt-induced myocardial dysfunction although a mechanism was not identified. Significant effects on cardiac function were not observed following 16 weeks of exposure. In guinea pigs, exposure to 20 mg cobalt/kg-day as cobalt sulfate in the diet for 5 weeks resulted in decreased absolute and relative heart weights and a greater incidence of abnormal electrocardiograms compared to animals fed on diets not supplemented with cobalt (Mohiuddin et al., 1970). Cardiac arrhythmias, including bradycardia, and repolarization abnormalities, were observed in 65% of cobalt-treated animals compared to 5% of control animals. Cellular alterations, observed at the light and electron microscopic levels, in cardiac tissues included pericardial thickening and inflammation, myocardial degeneration and vacuolization, endocardial thickening and myofibrillar damage. In contrast, no effects on cardiac function were observed in male rats (12/group) exposed to protein-restricted diets containing 8.4 mg cobalt/kg-day for 8 weeks (Pehrsson et al., 1991). Treated rats showed a significant decrease in body weight but no differences in left ventricular function relative to animals treated with protein-restricted diets without added cobalt.

Histopathological changes in the thyroid gland have been observed following exposure of mice to 400 ppm cobalt chloride in drinking water (26 mg cobalt/kg-day) for 15 to 45 days (Shrivastava et al., 1996). The severity of effect increased with exposure duration. After 15 days of exposure, a reduction in thyroid epithelial cell height with degenerated nuclei and reduced amount of colloid with peripheral resorption vacuoles was observed, with more

pronounced effects after 30 days of exposure. More significant degenerative changes were observed after 45 days of exposure, including necrotic epithelial cells, reduced connective tissue between follicles, lymphocytic infiltrate and larger amounts of colloid within the lumen.

Developmental effects of orally administered cobalt have been studied in rats, rabbits and mice (Szakmary et al., 2001; Pedigo and Vernon, 1993; Paternain et al., 1988; Seidenberg et al., 1986; Domingo et al., 1985). Szakmary et al. (2001) evaluated the developmental effects of oral cobalt sulfate exposure in rats, mice and rabbits. Exposure of pregnant rats to 5.2-21.0 mg cobalt/kg-day (oral gavage) decreased perinatal growth and survival, retarded skeletal development and produced skeletal and urogenital malformations, with a LOAEL of 5.2 mg cobalt/kg-day. Maternal toxicity (increased relative liver, adrenal, spleen weights; increased BUN, serum creatinine) was only observed at the highest dose (21.0 mg cobalt/kg-day). Thus, embryotoxicity in rats was observed at exposure levels below the LOAEL for maternal toxicity. In pregnant mice exposed to 10.5 mg cobalt/kg-day, retarded skeletal development and malformations of the eye, kidney and skeleton were observed in the absence of maternal toxicity. In pregnant rabbits exposed to 4.2 mg cobalt/kg-day, 20% mortality was observed in dams. Fetal resorptions were observed in 30% of surviving dams. Results of the studies in rats and mice provide evidence that adverse developmental effects can occur in the absence of maternal toxicity.

Domingo et al. (1985) treated pregnant female rats (15 animals/group) with 5.4 to 21.8 mg cobalt/kg-day as cobalt chloride from gestation day 14 through lactation day 21. Offspring were examined for mortality, body weight, body and tail length and general signs of toxicity after 1, 4 and 21 days of nursing. In contrast to the study by Szakmary et al. (2001), results of the Domingo et al. (1985) study reported maternal toxicity at all doses that produced adverse developmental effects (specific maternal effects observed were not reported). Fetal effects at 5.4 mg cobalt/kg-day included stunted growth of the pups of both sexes, decreased body length and tail length in male offspring and decreased spleen and liver weight in female offspring. Effects at the 10.9 mg cobalt/kg-day dose included decreased body weight in female pups, while at 21.8 mg cobalt/kg-day, decreased number of living young and decreased survival were seen. Blood parameters (liver enzymes, bilirubin, total protein, uric acid, urea, creatinine, hemoglobin and hematocrit) in pups did not show any treatment-related changes. No signs of toxicity were observed in surviving pups in any of the cobalt exposure groups.

No significant effects on fetal growth or survival were found in rats exposed to 6.2 to 24.8 mg cobalt/kg-day as cobalt chloride (oral gavage) during gestation days 6-15 (Paternain et al., 1988). The incidence of stunted fetuses was higher in the animals treated with 12.4 or 24.8 mg cobalt/kg-day (0.3 stunted fetuses per litter in the 12.4 mg cobalt/kg-day group; 1.0 stunted fetuses per litter in the 24.8 mg cobalt/kg-day group) compared to the control group (0 stunted fetuses per litter); however, the differences were not statistically significant. No treatment-related effects were observed for the number of corpora lutea, total implants, resorptions, the number of dead and live fetuses or fetal size parameters. No gross external abnormalities, skeletal malformations or other signs of fetal toxicity were observed. Maternal effects, including reduced body weight gain and food consumption and altered hematological parameters

(increased hematocrit, hemoglobin and reticulocytes), were reported at all exposure levels. No fetal effects were reported in mice exposed to 81.7 mg cobalt/kg-day (oral gavage) during gestation days 8-12 (Seidenberg et al., 1986), but a significant ($p<0.05$) decrease in maternal weight was found. Additional details were not reported.

Pedigo and Vernon (1993) exposed male rats to 93 mg cobalt/kg-day as cobalt chloride in the drinking water for 10 weeks, after which the males were mated with control females to examine for dominant lethal effects. Relative to the control group, the cobalt treatment group had a lower percentage of pregnant females (control, 29/32; cobalt, 18/31), lower number of implantations per female (control, 8.3; cobalt, 6.5) and higher preimplantation losses (control, 0.43; cobalt, 2.4). At the end of the 10-week treatment period, sperm concentration was decreased to 15.3% and motility decreased to 18.3% of controls. Several measures of sperm velocity were also depressed relative to controls. All sperm parameters, except sperm concentration, returned to control levels 8 weeks after the cobalt exposure was terminated. The increase in preimplantation losses in the dominant lethal assay appears related to adverse effects on spermatogenesis rather than to effects on preimplantation development of embryos.

Several studies reported testicular degeneration and atrophy in rats exposed to 6.1 to 24.4 mg cobalt/kg-day as cobalt chloride for 2-3 months in the diet or in the drinking water (Anderson et al., 1992, 1993; Pedigo et al., 1988; Corrier et al., 1985; Mollenhauer et al., 1985; Domingo et al., 1984; Nation et al., 1983). Pedigo et al. (1988) exposed male CD-1 mice to 100, 200 or 400 ppm of cobalt chloride (~6.1, 12.2 or 24.4 mg cobalt/kg-day, respectively) in the drinking water for 13 weeks. High-dose animals showed a significantly decreased testicular weight beginning at week 9 of treatment and a decreased epididymal sperm concentration by week 11 of treatment. All dose groups showed significantly decreased testicular weight and epididymal sperm concentration and increased serum testosterone levels by week 12 of exposure, with the magnitude increasing with dose. Effects on serum testosterone levels may be secondary to effects on spermatogenesis and related to inhibition of local inhibitory feed-back mechanisms.

Anderson et al. (1992, 1993) exposed groups of male CD-1 mice to 400 ppm of cobalt chloride (~24.4 mg cobalt/kg-day) in the drinking water for up to 13 weeks. A decrease in testicular weight and a progressive degeneration of the seminiferous tubules were seen beginning at 9 weeks of exposure. Initial changes were vacuolization of Sertoli cells and abnormal spermatid nuclei, followed by sloughing of cells, shrinkage of tubules and thickened endothelium. No recovery was reported after a 20-week non-exposure recovery period. Co-administration of 800 ppm of zinc chloride provided a partial protection against the effects of cobalt. Similar histology (degeneration of the testes, particularly the seminiferous tubules) was noted in Sprague-Dawley rats exposed to 20 mg cobalt/kg-day in the diet for up to 98 days (Corrier et al., 1985; Mollenhauer et al., 1985). Decreased testicular weight was seen in Sprague-Dawley rats exposed to 500 ppm cobalt chloride (~17 mg cobalt/kg-day) for 3 months (Domingo et al., 1984).

Nation et al. (1983) exposed groups (n=6) of male Sprague-Dawley rats (weighing 200-210 g) to diets containing 0, 5 or 20 mg cobalt/kg-day for a total of 69 days. Following 14

days of exposure, animals were trained for scheduled (operant) or conditioned suppression neurobehavioral tests. Other than two seizures in the same high-dose animal, no overt signs of neurotoxicity were reported at any exposure level. A trend toward a decreased response rate in the schedule training behavior was observed in both the exposed groups but only attained statistical significance in the high-dose animals near the end of the operant testing period (sessions 28-35, on exposure days 44-51). A trend toward decreased conditioned suppression behavior did not attain statistical significance in either group. Animals exposed to 20 mg cobalt/kg-day, but not 5 mg cobalt/kg-day, showed a significantly decreased weight of the testes following 69 days of exposure. This study established a NOAEL of 5 mg cobalt/kg-day and a LOAEL of 20 mg cobalt/kg-day for decreased testicular weight and changes in operant behavior in male Sprague-Dawley rats.

Several other studies have examined the effects of cobalt on neurobehavioral parameters (Singh and Junnarkar, 1991; Krasovskii and Fridlyand, 1971; Bourg et al., 1985). In groups of male Sprague-Dawley rats (n=8) exposed to 20 mg cobalt/kg-day as cobalt chloride for 57 days in the drinking water, cobalt enhanced behavioral reactivity to stress (the animals were less likely to descend from a safe platform to an electrified grid) (Bourg et al., 1985). Singh and Junnarkar (1991) reported a moderate reduction in spontaneous activity and mild hypothermia in rats exposed orally to cobalt chloride (approximately 8 mg cobalt/kg-day) or cobalt sulfate (approximately 35 mg cobalt/kg-day). Krasovskii and Fridlyand (1971) exposed groups of rats (number and sex not specified) to 0.05, 0.5 or 2.5 mg cobalt/kg-day for up to 7 months. Neurobehavioral tests showed that treatment with cobalt as cobalt chloride resulted in a significant ($p<0.05$) increase in the latent reflex period at 0.5 mg cobalt/kg and above, and a pronounced neurotropic effect (disturbed conditioned reflexes) at 2.5 mg cobalt/kg.

Inhalation Exposure

In a subchronic inhalation study, groups of 10 F344/N rats and 10 B6C3F1 mice of each sex were exposed to cobalt sulfate hexahydrate aerosol (MMAD=0.83-1.10 μm; σ_g not reported) at concentrations of 0, 0.3, 1, 3, 10 or 30 mg/m^3 (0, 0.067, 0.22, 0.67, 2.2 or 6.7 mg cobalt/m^3) 6 hours/day, 5 days/week for 13 weeks (Bucher et al., 1990; NTP, 1991). Although this report indicates that exposure was to cobalt sulfate heptahydrate aerosol, detailed analysis of the cobalt aerosol in the 2-year continuation study (Bucher et al., 1999; NTP, 1998) reports that the aerosol was actually composed of cobalt sulfate hexahydrate; thus, exposure to the hexahydrate form is assumed for the 13-week study. Animals were monitored for body weight and observed for clinical signs during the exposure period. Urine samples for urinalysis and cobalt determination were collected from rats prior to sacrifice. Following termination of exposure, all animals were sacrificed and necropsied. Blood samples were collected and analyzed for hematological parameters (rats and mice) and serum chemistry and thyroid function parameters (rats only). The major organs were weighed. Animals from the control and high-dose groups received comprehensive histopathological examinations, while those from the lower dose groups received more limited examinations focused on the respiratory tissues.

All rats survived until scheduled necropsy (NTP, 1991; Bucher et al., 1990). Gross evidence of toxicity was noted only in rats exposed to 30 mg/m^3, and they displayed clinical signs of toxicity (ruffled fur, hunched posture) and reduced body weights. Polycythemia, indicated by significant increases in red blood cell count, hemoglobin and hematocrit, was noted in males exposed to ≥3 mg/m^3 and females exposed to ≥10 mg/m^3. In addition, platelets were significantly reduced in rats of both sexes at ≥10 mg/m^3 and reticulocytes were increased in females at 30 mg/m^3. Leukocyte counts and differentials were unaffected. Serum cholesterol was significantly reduced in males at ≥10 mg/m^3 and females at 30 mg/m^3. No other serum chemistry parameters were affected, including creatine kinase isozymes indicative of damage to cardiac muscle cells. Among the thyroid hormones, T3 (triiodothyronine) was significantly reduced in females at 10 mg/m^3 (83% of control) and males at 30 mg/m^3 (62% of control) and TSH (thyrotropin) was significantly reduced in males at 30 mg/m^3 (30% of control), but T4 (thyroxin) was not affected in either sex at any dose and the researchers concluded that thyroid function was not consistently affected in this study. Urinalysis revealed a dose-related increase in the number of epithelial cells and granular casts in the urine of many exposed male rats (3-7 per group exposed to ≥3 mg/m^3) but no controls in the urine of males rats. The researchers interpreted this finding as indicating minimal nephropathy in exposed male rats although histopathological lesions were not detected in the kidney. No effects on sperm counts, sperm motility or the incidence of abnormal sperm were noted. Average estrus cycle of females exposed to 30 mg cobalt/m^3 was slightly longer than controls, but the difference was not significant. Absolute and relative lung weights were significantly increased in both male and female rats at ≥1 mg cobalt/m^3. Other organ weights were not affected by treatment. Compound-related lesions were found only in the respiratory tissues of exposed rats. Degenerative, inflammatory and regenerative lesions were found throughout the respiratory tract (Table 2). Incidence and severity of lesions were similar in males and females. The most sensitive tissue was the larynx, with squamous metaplasia present at all exposure levels.

Among mice, 2/10 males exposed to 30 mg cobalt/m^3 died during the study (NTP, 1991; Bucher et al., 1990). The only clinical signs of toxicity observed were rapid breathing and skin discoloration in one of the mice that died. Body weights were reduced throughout the study in both males and females exposed to 30 mg/m^3. No dose-related hematological effects were found. Absolute and relative lung weights were significantly increased in male and female mice exposed to ≥10 mg/m^3. Respiratory lesions were similar to those observed in rats. As with rats, the most sensitive tissue was the larynx, with squamous metaplasia present at all exposure levels. Reproductive system effects were more prominent in mice than rats. Males had significantly decreased testicular weight (48% compared to control), decreased epididymal weight (81% compared to control), testicular atrophy consisting of loss of germinal epithelium in the seminiferous tubules and foci of mineralization and an increased percentage of abnormal sperm at 30 mg/m^3 (295% compared to control). Significant reductions in sperm motility of 90, 87 and 54% were observed in the 3, 10 and 30 mg/m^3 exposure groups, respectively (lower doses were not tested). Females had a significantly increased length of the estrus cycle at 30 mg/m^3 (119% longer compared to control).

Table 2. Rats with Selected Lesions in the 13-Week Inhalation Study with Cobalt Sulfate (NTP, 1991; Bucher et al., 1990)

SITE	LESION	EXPOSURE GROUP					
		Control	0.3 mg/m^3	1 mg/m^3	3 mg/m^3	10 mg/m^3	30 mg/m^3
Larynx	Inflammation	M: 0 F: 1	M: 2 F: 2	M: 8 ** F: 7 **	M: 9 ** F: 10 **	M: 9 ** F: 10 **	M: 9 ** F: 10 **
	Squamous metaplasia	M: 0 F: 1	M: 9 ** F: 7 **	M: 10 ** F: 10 **	M: 10 ** F: 10 **	M: 10 ** F: 10 **	M: 10 ** F: 10 **
Lung	Inflammation	M: 0 F: 0	M: 0 F: 0	M: 6 * F: 2	M: 10 ** F: 9 **	M: 10 ** F: 10 **	M: 10 ** F: 10 **
	Fibrosis	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0	M: 0 F: 1	M: 1 F: 4 *	M: 10 ** F: 5*
	Bronchiolar epithelium regeneration	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0	M: 7 ** F: 5 *

M: number of males with lesions
F: number of females with lesions
* $p<0.05$ vs controls by Fisher exact test
** $p<0.01$ vs controls by fisher exact test

Other studies in animals have also reported respiratory lesions and altered respiratory function following inhalation exposure to cobalt. Kyono et al. (1992) observed mild pulmonary lesions in rats exposed to 2.12 mg/m^3 of cobalt aerosols 5 hours/day for 4 days. Lesions were characterized by focal hypertrophy of the epithelium, abnormal macrophages, vacuolization of type I epithelial cells and proliferation of type II epithelial cells, which are indicative of an initial inflammatory response. Kerfoot et al. (1975) exposed groups of five miniature swine to 0, 0.1 or 1.0 mg/m^3 of cobalt dust for 6 hours/day, 5 days/week for 3 months. Wheezing was observed in animals from both cobalt groups after 4 weeks of exposure (numeric data not reported). Tidal volume was decreased to 73% and 64% of controls in the low and high dose groups, respectively, and total respiratory compliance was decreased relative to controls (low dose, 66% of control; high dose, 56% of control). Statistical significance was not reported. Examination of lung tissue by electron microscopy revealed septa thickened by collagen, elastic tissue and fibroblasts in both exposure groups, with more pronounced effects in the high dose group. Johansson et al. (1987) exposed rabbits (8/group) to 0.4 or 2 mg cobalt/m^3, 6 hours/day, 5 days/week for 14-16 weeks. Nodular accumulation of alveolar type II cells (8/8 rabbits in both cobalt groups), abnormal accumulation of enlarged, vaculolated alveolar macrophages (5/8 in the

low dose group and 8/8 in the high dose group) and interstitial inflammation (4/8 rabbits in the low dose group and 8/8 rabbits in the high dose group) were observed, with more pronounced effects in the high dose group.

The carcinogenicity of inhaled cobalt was investigated in groups of 50 F344/N rats and 50 B6C3F1 mice of each sex exposed to cobalt sulfate hexahydrate aerosol (MMAD=1.4-1.6 μm; σ_g=2.1-2.2) at concentrations of 0, 0.3, 1 or 3 mg/m^3 (0, 0.067, 0.22 or 0.67 mg cobalt/m^3) 6 hours/day, 5 days/week for 105 weeks (Bucher et al., 1999; NTP, 1998). Animals were monitored for body weight and observed for clinical signs during the exposure period. Following termination of exposure, all animals were sacrificed and necropsied. At necropsy, all organs and tissues were examined for gross lesions, trimmed and examined histologically.

In F344 rats, there were no changes in survival or mean body weights in males or females of any exposure group (Bucher et al., 1999; NTP, 1998). Irregular breathing was noticed more frequently in female rats exposed to 3 mg cobalt/m^3 than in controls or other groups; no changes in clinical signs were noted in any of the treated male rats. Incidence of selected neoplasms and nonneoplastic lesions of the lung in rats is summarized in Table 3. Both male and female rats in all exposure groups showed a high incidence (94% or greater) of squamous metaplasia of the alveolar epithelium, fibrosis of the pulmonary interstitium and granulomatous inflammation, with all lesions increasing in severity with increasing exposure level. Significant increases in alveolar/bronchiolar adenomas or carcinomas were seen in high-dose male rats, while significant increases in alveolar/bronchiolar adenomas or carcinomas were seen in the mid- and high- dose female rats. The combined incidence of alveolar/bronchiolar neoplasms (adenoma and carcinoma) in male rats and female rats was significantly greater than that in control animals, and a significant linear trend occurred in both sexes. Rats of both sexes showed treatment-related increases in hyperplasia of the lateral nasal wall, atrophy of the olfactory epithelium and squamous metaplasia of the larynx. A significant increase in the incidence of pheochromocytoma in 3 mg cobalt/m^3 dosed females was also noted (2/48, 1/49, 4/50 and 10/50 in control, 0.3, 1 and 3 mg cobalt/m^3 groups, respectively). A marginally increased incidence of pheochromocytoma in males exposed to 1 mg/m^3, but not in those exposed to 3 mg/m^3, was considered by the study authors not to be related to treatment.

Table 3. Incidence of selected neoplasms and nonneoplastic lesions in the respiratory tract of rats in the 2-year inhalation study of cobalt sulfate (Bucher et al., 1999; NTP, 1998)

SITE	LESION TYPE	EXPOSURE GROUP			
		Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Lung	Alveolar epithelium hyperplasia	M: 9 F: 15	M: 20 * F: 7	M: 20 * F: 20	M: 23 ** F: 33 **
	Alveolar epithelium metaplasia	M: 0 F: 2	M: 50 ** F: 47 **	M: 48 ** F: 50 **	M: 49 ** F: 49 **
	Inflammation granulomatous	M: 2 F: 9	M: 50 ** F: 47 **	M: 48 ** F: 50 **	M: 50 ** F: 49 **
	Alveolar/bronchiolar adenoma	M: 1 F: 0	M: 4 F: 1	M: 1 F: 10 **	M: 6 F: 9 **
	Alveolar/bronchiolar carcinoma	M: 0 F: 0	M: 0 F: 2	M: 3 F: 6 *	M: 1 F: 6*
	A/B adenoma or carcinoma	M: 1 F: 0	M: 4 F: 3	M: 4 F: 15 **	M: 7 * F: 15 **
	Squamous cell carcinoma	M: 0 F: 0	M: 0 F: 0	M: 0 F: 1	M: 0 F: 1
Nose	Lateral wall hyperplasia	M: 2 F: 1	M: 14 ** F: 8 *	M: 21 ** F: 26 **	M: 21 ** F: 38 **
	Olfactory epithelium atrophy	M: 8 F: 5	M: 24 ** F: 29 **	M: 42 ** F: 46 **	M: 48 ** F: 47 **
Larynx	squamous metaplasia	M: 0 F: 1	M: 10 ** F: 22 **	M: 37 ** F: 39 **	M: 50 ** F: 48 **

M: Incidence of lesions in male rats
F: Incidence of lesions in female rats
* $p<0.05$ compared to control by logistic regression test
** $p<0.01$ compared to control by logistic regression test

In B6C3F1 mice, no changes in survival were observed in any exposure group (Bucher et al., 1999; NTP, 1998). Male mice exposed to 3 mg cobalt/m^3 showed a decreased mean body weight relative to controls from week 96 through the end of the study (105 weeks). Mean body weights of exposed female mice were generally greater than those of controls throughout the study. Irregular breathing was noted slightly more frequently in female mice exposed to 1 mg/m^3 than in controls or other exposed groups. Incidence of selected neoplasms and nonneoplastic lesions of the lung in mice is summarized in Table 4. A dose-related increase in the occurrence of cytoplasmic vacuolization of the bronchus was seen in both sexes of mice, with incidences at all exposure levels being significantly different from controls. As in rats, both sexes of mice showed a significant linear trend toward increased alveolar/bronchiolar tumors, with the 3-mg/m^3 male and the 1- and 3- mg/m^3 female groups attaining statistical significance. Mice of both sexes showed significantly increased incidences of squamous metaplasia of the larynx ($p<0.05$) at all exposure levels examined. In male mice, but not in females, the incidence of hemangiosarcoma was significantly elevated in animals exposed to 1 mg/m^3, but not in other

Table 4. Incidence of selected neoplasms and nonneoplastic lesions in the respiratory tract of mice in the 2-year inhalation study of cobalt sulfate (Bucher et al., 1999; NTP, 1998)

SITE	LESION TYPE	EXPOSURE GROUP			
		Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
	Bronchus cytoplasmic vacuolization	M: 0 F: 0	M: 18 ** F: 6 *	M: 34 ** F: 31 **	M: 38 ** F: 43 **
	Alveolar/bronchiolar adenoma	M: 9 F: 3	M: 12 F: 6	M: 13 F: 9	M: 18 * F: 10 *
	Alveolar/bronchiolar carcinoma	M: 4 F: 1	M: 12 F: 1	M: 13 F: 4	M: 18 * F: 9 **
	A/B adenoma or carcinoma	M: 11 F: 4	M: 14 F: 7	M: 19 F: 13 **	M: 28 ** F: 18 **
Nose	Olfactory epithelium atrophy	M: 0 F: 0	M: 0 F: 2	M: 28 ** F: 12 **	M: 48 ** F: 46 **
	hyperplasia	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0	M: 10 ** F: 30 **
Larynx	Squamous metaplasia	M: 0 F: 0	M: 37 ** F: 45 **	M: 48 ** F: 40 **	M: 44 ** F: 50 **

M: Incidence of lesions in male rats
F: Incidence of lesions in female rats
* $p<0.05$ compared to control by logistic regression test
** $p<0.01$ compared to control by logistic regression test

exposure groups (2/50, 4/50, 8/50 and 7/50 in the control, 0.3, 1 and 3 mg/m^3 groups, respectively).

Wehner et al. (1977, 1979) exposed 2-month-old male Syrian golden hamsters to inhaled cobalt oxide at 0 or 10 mg/m^3 (51 animals/group), 7 hours/day, 5 days/week for approximately 15 months. The incidence of tumors in treated hamsters was not statistically different from controls. There was "limited" histopathologic and ultrastructural examination in the study.

No developmental toxicity studies were located following inhalation exposure to cobalt.

Other Studies

Parenteral Administration

Heath (1956) injected groups of 10 male and 20 female rats with a single intramuscular 28 mg dose of powdered cobalt in the thigh. Injection-site sarcomas appeared in 18 (60%) of the treated rats within 5-12 months. Similar results were observed in Wistar rats by Gilman (1962) and Gilman and Ruckerbauer (1962), with single intramuscular doses of 20 mg of cobalt oxide

and cobalt sulfide. Cobalt oxide and cobalt sulfide given intramuscularly at doses twice those used in rats did not induce sarcomas in mice (Gilman and Ruckerbauer, 1962). Shabaan et al. (1977) observed a high incidence of fibrosarcomas in rats given subcutaneous injections of cobalt chloride at 40 mg/kg-day for 10 days. Tumors developed in 8-12 months. Stoner et al. (1976) tested cobalt acetate in the strain A mouse pulmonary tumor test. Groups of 20 mice/sex received three times per week intraperitoneal injections for a total of 19 cumulative doses of 0, 95, 237 or 475 mg/kg. Survival was high over the 30-week observation period, and the incidence of lung tumors in treated mice was not statistically different from controls.

Genotoxicity Studies

The genetic toxicity of cobalt was reviewed by Beyersman and Hartwig (1992) and more recently by De Boeck et al. (2003b), Hartwig and Schwerdtle (2002) and Lison et al. (2001). Cobalt compounds have generally tested negative in bacterial mutagenicity assays, with occasional positive results occurring only with the addition of an exogenous metabolic system. In contrast, cobalt compounds have generally tested positive in yeast and plant cells. In mammalian cell systems, cobalt has been shown to induce DNA strand breaks, sister-chromatid exchanges and morphological cell transformation.

Results of *in vitro* studies using human peripheral blood mononucleated cells show that cobalt metal and cobalt chloride induced DNA strand breaks at non-cytotoxic concentrations (De Boeck et al., 1998, 2003a). Evidence demonstrating mutagenic activity of cobalt *in vivo* in humans is lacking. No significant change in DNA strand breaks were observed in lymphocytes from nonsmoking workers who had been occupationally exposed to cobalt or hard metal dust although a positive association was observed between DNA strand breaks and smoking (De Boeck et al., 2000).

Experimental data in animals provide evidence of genotoxicity following *in vivo* exposure to cobalt. Single oral exposure of male Swiss mice to 0, 4.96, 9.92 or 19.8 mg cobalt/kg-day, as cobalt chloride, resulted in significantly increased percentages of both chromosomal breaks and chromosomal aberrations in bone marrow cells, with significant linear trends toward increasing aberrations with increased exposure (Palit et al., 1991a,b,c,d). Thirty hours following single intraperitoneal injection of cobalt(II) chloride in BALB/c mice, an increase in micronucleus formation was seen in mice injected with 12.4 or 22.3 mg cobalt/kg (as cobalt chloride) but not in mice injected with 6.19 mg cobalt/kg (Suzuki et al., 1993). Single injection of 12.4 mg/kg $CoCl_2 \cdot 6\ H_2O$ resulted in significantly increased micronucleus formation at 24 hours post-injection but not at 12, 48, 72 or 96 hours. Pedigo and Vernon (1993) reported that treatment with 400 ppm cobalt (99 mg cobalt/kg-day) in the drinking water of mice for 10 weeks resulted in an increase in dominant lethal effects as indicated by changes in the number of pregnant females, percentage of live embryos and number of pre-implantation losses per female.

While the mode of action of cobalt has not been determined, the generation of cobalt-induced oxidative stress and DNA repair inhibition may be involved. Exposure to cobalt compounds increases indices of oxidative stress, including diminished levels of reduced

glutathione, increased levels of oxidized glutathione, increased levels of oxygen radicals and increased free-radical-induced DNA damage (Kawanishi et al., 1994; Lewis et al., 1991; Kadiiska et al., 1989; Zhang et al., 1998; Moorehouse et al., 1985). A recent review by Lison et al. (2001) concludes that results of *in vitro* and *in vivo* experiments in animal models indicate that two different mechanisms of genotoxicity appear to contribute to the carcinogenic potential of cobalt compounds: DNA breakage and inhibition of DNA repair. A review by Hartwig and Schwerdtle (2002) concludes that cobalt may specifically target zinc finger structures in DNA repair proteins, interfering with base and nucleotide excision repair.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR COBALT

Indicators of adverse human health effects following oral exposure to cobalt (Co) include increased erythrocyte production, raised hemoglobin levels, decreased iodine uptake by the thyroid gland, elicitation of dermatitis in sensitized individuals and cardiomyopathy. Observations in humans for effects on the heart, blood and the thyroid gland are supported by results of studies in animals. Other effects, including neurobehavioral, developmental and testicular toxicity were observed only in animals and at relatively high doses; we did not consider these critical endpoints for this risk assessment.

Cardiomyopathy is an endpoint of concern for cobalt in humans; however, it is probable that alcohol consumed in "beer-cobalt cardiomyopathy," as well as other factors, played a role in the effects that we saw. Therefore, we could not determine a dose-response relationship for cobalt from the human studies. Studies in animals have noted cardiac effects following cobalt exposure at higher exposure levels than observed in human studies of "beer-cobalt cardiomyopathy." On this basis, we did not select cardiomyopathy as the critical endpoint for p-RfD derivation.

We evaluated the elicitation of an allergic response in cobalt-sensitized workers as a potential critical endpoint for the derivation of an oral p-RfD. However, the available data provide no information on the dose-response relationship of cobalt sensitization, nor is a no observable adverse effect level (NOAEL) for the elicitation of the allergic response in humans defined. Interrelationships also exist between cobalt and nickel (Ni) sensitization so that people sensitized by (Ni) may have an allergic reaction following cobalt exposure. We, therefore, did not select sensitization as the critical endpoint for p-RfD derivation.

Adverse effects of cobalt on the thyroid gland were identified as an endpoint of concern based on a preliminary report by Roche and Layrisse, 1956. This report showed that oral exposure for 2 weeks markedly inhibited iodine uptake in humans by the thyroid gland at an exposure level of 1 mg cobalt/kg-day. Additionally, reduced iodine uptake has also been reported in a small clinical study where 2 out of 4 patients were exposed to 0.54 mg cobalt/kg-day (Paley et al., 1958). Further review of this report indicated that one of the two subjects reported to have reduced iodine uptake had received intravenous (i.v.) cobalt in addition to oral

cobalt intake. The i.v. dosing may have raised the internal concentration to a level greater than the estimated 0.54 mg cobalt /kg-day intake associated with reduced iodine uptake. Details of the clinical conditions are not available; thus we are currently unable to ascertain the mechanism by which cobalt reduces iodine uptake. Smith (2000) reported inflammation and necrosis of the thyroid gland in mice following oral exposure to cobalt, although they observed the effects at a higher exposure level than those that were reported to affect thyroid function in humans. In humans, thyroid effects and cobalt-induced polycythemia appear to occur at similar daily exposure levels (value). Furthermore, reduced iodine uptake as a critical effect of cobalt exposure will result in p-RfDs lower than the daily dietary exposure which is critical for vitamin B_{12}.

Cobalt has been shown to increase erythrocyte production number and hemoglobin levels through stimulation of erythropoietin, a hormone produced primarily in the kidney. Hematological effects of cobalt treatment (increased hemoglobin and erythrocyte number) have been reported in healthy, non-anemic adults (Davis and Fields, 1958) and in anemic anephric dialysis patients (Taylor et al., 1977; Duckham and Lee, 1976). In these anephric dialysis patients, treatment with cobalt resulted in an increase in hemoglobin from levels clinically described as "anemic" to levels at or near "normal." Thus, the effect of cobalt administration in these patients was clinically beneficial -- not adverse. Results of these studies are difficult to interpret due to confounding factors, including the anephric status of patients and the concomitant administration of iron. Hematologic effects of cobalt were also found in several studies in rats (Domingo et al., 1984; Krasovskii and Fridlyand, 1971; Murdock, 1959; Holly, 1955, Stanley et al., 1947), supporting the plausibility for the effects observed in humans.

This manuscript presents two possible approaches for the the derivation of p-RfDs for subchronic and chronic durations..

First Approach:

Davis and Fields (1958) reported hemoglobin level increases of 6-11% over "normal" in healthy volunteers given 0.97 mg cobalt/kg-day as cobalt chloride. Their study was used as the critical study for derivation of the subchronic and chronic p-RfDs. Although increases in hemoglobin levels and erythrocyte production are beneficial effects in anemic patients, polycythemia in non-anemic individuals is an adverse effect since related increases in blood viscosity can lead to exacerbation of hypertension, blood clots, heart attacks and strokes (Hillman, 2001). An increased risk of polycythemia-related cardiovascular morbidity and mortality is associated with hematocrits greater than 40% (Hillman, 2001). Thus, we expect the effects related to the relatively small increases in hemoglobin levels and erythrocyte number observed in the Davis and Fields (1958) study to be subclinical in healthy individuals. Although Duckman and Lee (1976) reported a lower LOAEL (0.16 mg cobalt/kg-day) , we did not consider their value suitable for use due to confounding experimental factors, such as the simultaneous treatment with ferrous sulfate. We did not consider the study by Taylor et al. (1977) because it was poorly reported and did not present numeric data or statistical analyses of the outcomes. As a vital component of vitamin B_{12}, cobalt intake is very critical in young

children and adult human beings on vegetarian diets to maintain normal hematopoiesis. Furthermore, the LOAEL of 0.97 mg cobalt/kg-day reported for polycythemia is essentially comparable to the LOAEL of 1 mg cobalt/kg-day for adverse effects on thyroid function in humans (Roche and Layrisse, 1956); thus, subchronic and chronic RfDs based on the study by Davis and Fields (1958) are protective for adverse thyroid effects.

The LOAEL of 0.97 mg cobalt/kg-day reported for polycythemia in non-anemic humans was for an exposure period of only 22 days and does not represent subchronic or chronic exposure durations. Based on a half-life of approximately 1.3 days (ICRP, 1993), it is likely that cobalt levels (in the human body) reach a quasi-steady-state within the first week of exposure. If the steady-state level of cobalt in the human body is the major determinant of the stimulation of erythrocyte production (as opposed to the time-integrated level), then the effect should not progress with longer exposure duration. However, we could not locate any information about the effects of subchronic or chronic exposure on the cobalt-stimulated erythropoietin response in humans, including any potential physiological compensatory actions or effects of exposure duration on magnitude of the response. Subchronic exposure of rats to oral cobalt resulted in increased hemoglobin level and erythrocyte number. No studies investigating the hematological effects of chronic oral exposure to cobalt are available.

We derived the subchronic provisional RfD (subchronic p-RfD) and chronic provisional RfD (chronic p-RfD), in the absence of a NOAEL, from the LOAEL of 0.97 mg cobalt/kg-day as follows:

Subchronic p-RfD

Dividing the LOAEL of 0.97 mg cobalt/kg-day by an uncertainty factor (UF) of 100 yields a **subchronic p-RfD of 0.01 mg cobalt/kg-day**. The UF of 100 is composed of three factors: 3 for use of a LOAEL for effects considered to be of minimal severity, 3 for database insufficiencies and 10 for human variability. We chose the factor of 3 for a LOAEL considered to be of minimal severity since the effects associated with mild polycythemia are subclinical in healthy individuals. We included a factor of 3 for database uncertainties (absence of subchronic oral data), which lead to the use of a 22-day study for the subchronic p-RfD. No information is available to assess whether the severity of polycythemia changes as exposure duration increases. The factor of 10 accounts for human variability, including sensitive subgroups (individuals at risk of blood clots, strokes and heart attacks). Development of polycythemia in individuals with cardiovascular disease may result in the cardiovascular morbidity and mortality. This provisional subchronic RfD may not be protective for people with allergic hypersensitivity to cobalt.

Subchronic p-RfD = LOAEL ÷ UF
= 0.97 mg cobalt/kg-day ÷ 100
= **1×10^{-2} mg cobalt/kg-day**

Chronic p-RfD

Dividing the LOAEL of 0.97 mg cobalt/kg-day by an UF of 300 yields a **chronic p-RfD of 0.003 mg cobalt/kg-day**. The UF of 300 is composed of three factors: 3 for use of a LOAEL for effects considered to be of minimal severity, 10 for database insufficiencies and 10 for human variability. The factor of 3 was chosen for use of a LOAEL considered to be of minimal severity since the effects associated with mild polycythemia are subclinical in healthy individuals. A factor of 10 accounts for database uncertainties (absence of chronic or subchronic oral data) leading to the use of a 22-day study for the chronic p-RfD. No information is available to assess whether the severity of polycythemia changes as exposure duration increases from acute to lifetime exposure. The factor of 10 accounts for human variability, including sensitive subgroups (individuals at risk of blood clots, strokes and heart attacks). Development of polycythemia in individuals with cardiovascular disease may result in cardiovascular morbidity and mortality. This provisional chronic RfD may not be protective for people with hypersensitivity to cobalt.

Chronic p-RfD = LOAEL ÷ UF
= 0.97 mg cobalt/kg-day ÷ 300
= **3×10^{-3} mg cobalt/kg-day**

Confidence in the critical study is low-to-medium. The study examined six subjects over a 22-day exposure period. Since only a single dose level was evaluated, a NOAEL for effects on hematopoiesis was not established in the critical study. Studies in anemic humans and several studies in animals support the plausibility of cobalt producing the hematologic effects observed in the critical study and a mechanism of cobalt-induced polycythemia (stimulation of erythropoietin) has been established by Smith (1999). Confidence in the database is low-to-medium. There are no subchronic or chronic oral data in humans and no chronic oral data in animals. Studies in animals show that polycythemia is maintained over subchronic exposure durations. However, it is unknown whether cobalt-induced polycythemia is maintained over chronic exposure durations or how severity may change with increasing exposure duration. Furthermore, no information is available regarding the effects of chronic, mild polycythemia on cardiovascular function. Low-to-medium confidence in the provisional subchronic and chronic RfDs results.

The only known nutritional function of cobalt is as a vital component of vitamin B_{12}. All vitamin B_{12} is derived from bacterial synthesis so inorganic cobalt can be considered essential for animal species, such as ruminants, that depend totally on their bacterial flora for their vitamin B_{12}. This also may apply to humans on unsupplemented vegetarian diets, to some degree, whose intake of pre-formed vitamin B_{12} is severely limited. (Animal products are the primary source of

vitamin B_{12} in the diet.) However, there is no evidence that the intake of cobalt is ever limiting in the human diet and, therefore, no Recommended Daily Allowance (RDA) is deemed necessary for cobalt (NRC, 1989). Daily cobalt intakes have been estimated in two market basket studies yielding similar estimates (Dabeka and McKenzie, 1995; Pennington and Jones, 1987). Based on a 1984 Food and Drug Administration (FDA) Total Diet Study, daily cobalt intakes have been estimated to range from 3.4 to 11.6 μg cobalt for children aged 6 months to 14 years and from 6.2 to 10.8 μg cobalt for adults (Pennington and Jones, 1987). Using average age-, gender-category body weights from Tables 7-2 and 7-3 in the U.S. EPA Exposure Factors Handbook (U.S. EPA 1997b), we estimated the daily intakes based on the Pennington and Jones (1987) study for children to range from 0.14 to 0.39 μg cobalt/kg-day and for adults to range from 0.09 to 0.14 μg cobalt/kg-day (Appendix B). A Canadian Health and Welfare survey of Montreal estimated dietary intakes in the range of 7 to 14 μg cobalt for children aged 6 months to 19 years and 8 to 15 μg cobalt for adults (Dabeka and McKenzie, 1995). Based on body weights from the U.S. EPA Exposure Factors Handbook (U.S. EPA, 1997b), estimated daily intakes based on the Dabeka and McKenzie (1995) study for children range from 0.18 to 0.49 μg cobalt/kg-day and for adults range from 0.12 to 0.20 μg cobalt/kg-day (Appendix B). The recommended dietary allowance for Canadian infants is 0.012 μg/day of cobalt as vitamin B_{12} (ATSDR, 2004). The provisional subchronic RfD of 0.01 mg cobalt/kg-day (10 μg cobalt/kg-day) and the provisional chronic RfD of 0.003 mg cobalt/kg-day (3 μg cobalt/kg-day) are one order of magnitude (or more) higher than estimates of the daily intake of cobalt.

Second Approach:

Smith (2000) found that individuals who are exposed orally to 1mg cobalt/kg-day during a 2-week period experienced inhibited iodine uptake. A smaller clinical study, by Brown (1999), reported reduced iodine uptake in one out of two subjects treated with 0.54mg cobalt/kg-day for 10-14 days. Long-term exposure at 2-4 mg cobalt/kg-day in anemic children has been reported to cause goiter (Prescott et al., 1992). Additionally, thyroid toxicity in mice has been reported at 26 mg cobalt/kg-day during an exposure period of 15-45 days (Shrivastava et al., 1996). It is noteworthy to mention that the severity of effect (necrotic changes) increased with increased duration of exposure.

Subchronic provisional RfD

The reduced iodine uptake at 1mg cobalt/kg-day in humans is the LOAEL; dividing this by an UF of 300 (10 for LOAEL, 10 for inter-individual sensitivity and 3 for database uncertainty) would yield a **subchronic p-RfD of 0.003 mg/kg-day**. This p-RfD is about one order of magnitude lower than the p-RfD of 0.01 mg/kg-day, based on polycythemia.

Chronic provisional RfD

Using the above LOAEL of 1mg/kg-day, we could apply an additional factor of 10 for duration of exposure (Total UF: 3000) to derive a **p-RfD of 0.0003 mg/kg-day for chronic**

duration. This p-RfD is exactly one order of magnitude lower than the p-RfD of 0.003 mg/kg-day, based on polycythemia.

Confidence in the critical study is low-to-medium. Brown et al. (2000) examined twelve subjects over a two-week exposure period. Since they/we evaluated only a single dose level, a NOAEL for thyroid effects was not established in the critical study. Other human and animal studies support the plausibility of cobalt producing thyroid toxicity by inhibiting iodine uptake in exposed subjects. Confidence in the database is low-to-medium. There are no subchronic or chronic oral data for humans and animals. Although one study (Smith, 2000) of longer duration has reported increased severity of effects in thyroid glands of anemic children exposed at higher doses, details of this study are unavailable for assessment. We have low-to-medium confidence in the provisional subchronic and chronic RfDs results.

Recommendation:

Subchronic p-RfDs and chronic p-RfDsof 0.01 and 0.003 mg/kg-day, respectively, based on polycythemia, may not be protective for thyroid toxicity in long-term duration of exposure. Smith (2000) conducted a single dose mouse study and reported an increased severity of pathological lesions leading to necrotic changes in thyroid epithelia with increased duration of exposure. Furthermore, the chronic p-RfD of 0.0003 mg/kg-day would be slightly lower than the upper bound of the range for estimated daily intake (EDI) for cobalt in children (0.00039 mg/kg-day). As reported in the ATSDR Profile (2004), the daily requirement of cobalt for Vitamin B_{12} is approximately 0.00001 mg cobalt/kg-day and the average intake from food sources is in the range of 0.00007 to 0.0005 mg cobalt/kg-day without any adverse health consequences. Thus, we consider the subchronic p-RfD and chronic p-RfDs of 0.003 and 0.0003 mg/kg-day, respectively, to be protective for both polycythemia and thyroid toxicity without compromising daily intake from food sources.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR COBALT

Of the four epidemiology studies discussed above, the study by Nemery et al. (1992) provides the strongest basis for derivation of a p-RfC. Workers in this study were exposed to lower air concentrations of cobalt, over a much more constrained range of exposures than in the studies by Gennart and Lauwerys (1990) and Swennen et al. (1993). Using the values obtained from personal air samples from the Nemery et al. (1992) study, which would not have been possible using the Gennart and Lauwerys (1990) or Swennen et al. (1993) studies, we were able to derive a discrete NOAEL (5.3 $\mu g/m^3$) and LOAEL (15.1 $\mu g/m^3$). Furthermore, the Nemery et al. (1992) study demonstrated a dose-effect relationship on lung function and urinary cobalt-levels, after adjusting for effects of smoking and gender. We did not consider the Prescott et al. (1992) study for RfC derivation because evidence of an effect on the thyroid gland was marginal (22% increase in serum levels of T4) and respiratory endpoints were not investigated (i.e., it is not clear whether respiratory effects and thyroid effects occurred at similar exposure levels).

The human and animal database indicates that respiratory effects are sensitive endpoints of cobalt toxicity. Respiratory effects have been widely reported in workers exposed to cobalt, while effects on the thyroid and other tissues have not (ATSDR, 2004). In addition, Swennen et al. (1993) found only marginal evidence of thyroid effects (a 7% decrease in T3) and no evidence of cardiomyopathy or polycythemia in workers clearly displaying evidence of respiratory effects.

Animal data support the conclusion that the respiratory tract is the critical target for inhaled cobalt (NTP, 1991; Bucher et al., 1990; Wehner et al., 1977). Subchronic inhalation exposure to cobalt resulted in cytotoxicity and reparative proliferation in all regions of the respiratory tract in both rats and mice (NTP, 1991; Bucher et al., 1990). The NTP (1991) study further demonstrated that cobalt can produce testicular effects in male mice following inhalation exposure, but the effects were produced only at relatively high dose levels. Oral studies have also identified the testes as a target for cobalt toxicity. We were not able to locate any multi-generation reproduction studies dealing with inhalation or oral exposure. Although we were unable to locate any developmental toxicity studies following inhalation exposure to cobalt, oral studies provide evidence that high oral doses of cobalt may produce developmental effects in animals (Szakmary et al., 2001; Paternain et al., 1988; Domingo et al., 1985). However, evidence of developmental or reproductive effects in humans is lacking.

We identified decreased lung function as the critical effect for derivation of the subchronic and chronic p-RfCs. Assuming the personal air samples to be more representative of worker exposure than the area air samples, the study by Nemery et al. (1992) identified a NOAEL for cobalt of 5.3 $\mu g/m^3$ and a LOAEL of 15.1 $\mu g/m^3$ for effects on lung function. Although the LOAEL may be biased low due to inclusion of data from workshop #9, this does not affect the p-RfC derivation. We adjusted the NOAEL for occupational exposure to continuous exposure as follows:

$$5.3\ \mu g/m^3\ (10\ m^3/d\ /\ 20\ m^3/d)\ (5\ d\ /\ 7\ d) = 1.9\ \mu g/m^3$$

Using the $NOAEL_{ADJ}$ of 1.9 $\mu g/m^3$, we were able to derive the subchronic p-RfC and chronic p-RfC for cobalt as shown below.

Subchronic p-RfC

Dividing the $NOAEL_{ADJ}$ of 1.9 $\mu g/m^3$ by an UF of 30 yields a **subchronic p-RfC of $6x10^{-5}$ mg/m^3** for cobalt. The uncertainty factor of 30 is composed of two uncertainty factors: 3 for database insufficiencies and 10 for human variability. The factor of 3 was included for database insufficiencies due to the lack of inhalation developmental toxicity studies and a multi-generation reproduction study. One oral exposure study in rats, by Smith (2000) reports embryotoxic effects in the absence of maternal toxicity and several studies in animals provide evidence of testicular toxicity. The factor of 10 was included to account for human variability, including sensitive subgroups. Individuals with underlying respiratory diseases (asthma, chronic

obstructive pulmonary disease) may be more sensitive to the respiratory effects of inhaled cobalt. This subchronic p-RfC may not be protective for people with hypersensitivity to cobalt.

Subchronic p-RfC = $NOAEL_{ADJ} \div UF$
= $1.9\ \mu g/m^3 \div 30$
= **$6 \times 10^{-2}\ \mu g/m^3$**

Chronic p-RfC

Dividing the $NOAEL_{ADJ}$ of 1.9 $\mu g/m^3$ by an UF of 100 yields a **chronic p-RfC of 2×10^{-5} mg/m^3** for cobalt. The UF of 100 is composed of three uncertainty factors: 3 to account for uncertain exposure duration, 3 for database insufficiencies and 10 for human variability. The factor of 3 accounts for uncertain exposure duration in the critical study. Since Nemery et al. (1992) did not report duration for any worker in this study, it is possible that exposure duration may have been subchronic for some workers. The factor of 3 accounts for database insufficiencies due to the lack of inhalation developmental toxicity studies and a multi-generation reproduction study. One oral exposure study in rats (Smith, 2000) reports embryotoxic effects in the absence of maternal toxicity and several studies in animals provide evidence of testicular toxicity. The factor of 10 accounts for human variability, including sensitive subgroups. Individuals with underlying respiratory diseases (asthma, chronic obstructive pulmonary disease) may be more sensitive to the respiratory effects of inhaled cobalt. This chronic p-RfC may not be protective for people with hypersensitivity to cobalt.

Chronic p-RfC = $NOAEL_{ADJ} \div UF$
= $1.9\ \mu g/m^3 \div 100$
= **$2 \times 10^{-2}\ \mu g/m^3$**

Confidence in the key study is low because this cross-sectional study

- looked at only respiratory endpoints;
- included a control group that was studied more than 1 year after the exposed population;
- included a study group exposed to iron and diamond dust in addition to cobalt (and possibly to asbestos in the past);
- did not report duration of exposure; and
- encountered a number of procedural difficulties during its course (e.g., construction of control group).

Confidence in the database is medium. The choice of the critical endpoint is well supported by other studies in humans and animals. Subchronic exposure studies in rats and mice (NTP, 1991) found histopathological changes in the upper respiratory tract. Other studies in animals support these findings. Reproductive and developmental effects have not been adequately studied. We have medium-to-low confidence in the subchronic and chronic p-RfCs.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR COBALT

Weight-of-Evidence Classification

Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), cobalt is classified as "likely to be carcinogenic to humans by the inhalation route," based on both the limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in animals as shown by increased incidence of alveolar/bronchiolar tumors in both sexes of rats and mice (Bucher et al., 1999). While available studies in humans have suggested a possible association between exposure to cobalt and respiratory tumors in cobalt workers (Tuchsen et al., 1996; Mur et al., 1987; Morgan et al., 1983), limitations within these studies, including small numbers of subjects, inadequate exposure assessment and/or potential exposure to other chemicals make them inadequate for assessing the carcinogenic potential of cobalt. Available chronic animal studies have demonstrated the carcinogenic potential of inhaled cobalt in male and female rats and mice, with alveolar and bronchiolar tumors being the most prevalent (Bucher et al., 1999; NTP, 1998). We were unable to locate suitable studies for evaluation of the oral carcinogenic potential for cobalt were located.

Mode-of-Action Discussion

The U.S. EPA (2005a) Guidelines for Carcinogen Risk Assessment defines mode of action as "a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation." Examples of possible modes of carcinogenic action include mutagenic, mitogenic, anti-apoptotic (inhibition of programmed cell death), cytotoxic with reparative cell proliferation and immunologic suppression. Available evidence (Brown, 2000) suggests that bronchoalveolar tumors observed in animals following inhalation exposure to cobalt arise from genetic mechanisms although it is possible that tumors result from a proliferative response to injury in the respiratory tract.

Mutagenic Mode of Action

Key events

The precise mechanism of cobalt-induced carcinogenicity has not been fully determined. Smith (2000) suggests that cobalt is capable of eliciting genotoxic effects. While evaluations for mutagenic effects in bacteria have generally yielded negative results, results in several mammalian cell systems have suggested that cobalt is genotoxic in eukaryotic cells Smith (2000). Limited data from *in vivo* animal studies show that cobalt induces genotoxic effects, including chromosomal breaks, chromosomal aberrations and micronucleus formation. The most likely mechanisms for the genotoxic effects of cobalt are DNA breakage and the inhibition of DNA repair.

Strength, consistency, specificity of association

Although the carcinogenic potential of inhaled cobalt has been demonstrated in rats and mice by increased incidence of alveolar/bronchiolar tumors (Bucher et al., 1999; NTP, 1998), direct evidence demonstrating that cobalt can induce mutagenic changes in cells of the respiratory tract is lacking. *In vivo* exposure to hard metal dust containing 6.3% cobalt, 84% tungsten and 5.4% carbon induced DNA strand breaks in rat type II epithelial lung cells (De Boeck et al., 2003c). Smith (2000) observed chromosome/genome mutations within 12 hours of exposure to a single intratracheal instillation of 16.6 mg hard metal dust/kg body weight. Since the mutagenic potential of cobalt alone was not evaluated in this study, a causal relationship between type II epithelial cell mutations and cobalt exposure could not be established. Potential mutagenic changes in respiratory tract cells could also be mediated through activated oxygen species released by inflammatory cells (e.g., macrophages, polymorpohnuclear neutrophils), rather than directly by cobalt (Lison et al., 2001).

Dose-response concordance

A dose-response concordance has not been established between the development of bronchoalveolar tumors and mutagenesis following inhalation exposure to cobalt. Dose-response information on mutagenicity is available for acute oral and parenteral exposure to cobalt in mice (Suzuki et al., 1993; Palit et al., 1991a,b,c,d). No carcinogenicity data are available for the oral or parenteral routes upon which to base a dose-response concordance. Furthermore, we could not locate any data on the mutagenic potential of cobalt in respiratory tract cells following *in vitro* or *in vivo* exposure.

Temporal relationships

In vivo studies in animals show that acute oral and parenteral exposure to cobalt produces genotoxicity to bone marrow cells (Suzuki et al., 1993; Palit et al., 1991a,b,c,d). Due to the lack of data on the mutagenic potential of cobalt in respiratory tract cells, we could not assess the temporal relationship between potential mutagenic mechanisms and the development of bronchoalveolar tumors. Development of lung tumors in animals exposed to cobalt occurred following chronic exposure (NTP, 1998).

Biological plausibility and coherence

In vivo mutagenicity studies in mice show that oral and intraperitoneal exposure to single doses of cobalt chloride induced mutagenic changes in bone marrow cells (Suzuki et al., 1993; Palit et al., 1991a,b,c,d). Although it has been hypothesized that the bronchoalveolar tumors are the result of genotoxicity (De Boeck et al., 2003b; Hartwig and Schwerdtle, 2002; Lison et al., 2001), no direct evidence is available linking mutagenesis to the development of cancer. Carcinogenicity through an indirect mutagenic mode of action may also be mediated by activated inflammatory cells (macrophages, polymorpohnuclear neutrophils) (Lison et al., 2001).

Nongenotoxic Mode of Action

Key events

Cell proliferation in response to cell death from toxicity or other causes is a significant risk factor for cancer. Possible nongenotoxic modes of cobalt carcinogenic action include stimulation of cell proliferation (mitogenic) and cytotoxicity with subsequent reparative cell proliferation (cytotoxic). Regeneration of respiratory epithelial cells following injury from inhaled cobalt has the potential to produce carcinogenesis as a result of replication errors becoming fixed mutations before DNA repair can be completed.

Subchronic and chronic inhalation studies in rodents provide evidence that cobalt causes cell injury with subsequent reparative cell proliferation, suggesting that nongenotoxic actions may be involved in the development of bronchoalveolar tumors. Following inhalation exposure to cobalt sulfate aerosol (0.3 to 30 mg/m^3) for 3 months, rats and mice developed several lesions indicative of cell damage and proliferation throughout the entire respiratory tract, including nasal epithelial degeneration and metaplasia, laryngeal inflammation and metaplasia, bronchiolar epithelial regeneration and ectasia, alveolar hyperplasia and lung fibrosis (NTP, 1991; Bucher et al., 1990). Squamous hyperplasia of the larynx was the most sensitive effect (LOAEL=0.3 mg/m^3). The results of the 2-year carcinogenesis study in rats and mice revealed an increase in bronchoalveolar tumors in rats and mice (Bucher et al., 1999; NTP, 1998). Brown (2000) observed a statistically significant increase in combined alveolar/bronchiolar adenomas and carcinomas in the 3 mg/m^3 group but not in the 0.3 and 1 mg/m^3 groups for male rats and mice. In female rats and mice, Brown (2000) observed a statistically significant increase in combined alveolar/bronchiolar adenomas and carcinomas was observed in the 1 and 3 mg/m^3 groups but not in the 0.3 mg/m^3 group. These observations suggest the possibility that cell injury in the respiratory tract may have preceded the development of cancers although direct evidence for this assertion is lacking.

Strength, consistency, specificity of association

Inhaled cobalt produces cell damage with subsequent reparative cell proliferation and carcinomas and adenomas in the respiratory tract of rats and mice (Bucher et al., 1990, 1999; NTP, 1991, 1998). Although limited evidence of carcinogenicity in humans is available, results of several epidemioloic studies suggest a possible association between exposure to cobalt and respiratory tumors (Tuchsen et al., 1996; Mur et al., 1987; Morgan, 1983). Subchronic exposure studies in cobalt workers show an association between cobalt exposure and diminished pulmonary function (Nemery et al., 1992; Gennart and Lauwerys, 1990). Taken together, results of studies in rodents and humans suggest that inhaled cobalt produces a cytotoxic response in the respiratory tract that may contribute to decreases in pulmonary function and the development of bronchoalveolar tumors.

Dose-response concordance

Bucher et al. (1999) and NTP (1999) reported cobalt-induced bronchoalveolar tumors in rats and mice following chronic inhalation exposure. They observed tumors in male rats and mice in the 3 mg cobalt/m^3 exposure group and in female rats. Mice tumors were observed in the 1 and 3 mg/m^3 exposure groups. In this same study, granulomatous inflammation of the lung was observed at all exposure levels (0.3, 1 and 3 mg/m^3) in rats. Other markers of cell damage and proliferation, including hyperplasia, metaplasia and fibrosis, were observed in the 1 and 3 mg/m^3 exposure groups. Compared to rats, mice appeared to be less sensitive to cobalt-induced cytotoxic changes. Results of this study show that bronchoalveolar tumors develop at exposure levels that also produce cell damage and reparative proliferation although cell damage and repair are also observed at lower exposure levels than tumorigenesis.

Temporal relationships

Histopathological findings of the 3-month inhalation study in mice and rats show that inhaled cobalt induces cell damage and a reparative proliferative response throughout the entire respiratory tract (NTP, 1991; Bucher et al., 1990). The subsequent finding of bronchoalveolar tumors in the 2-year cancer bioassay is consistent with the hypothesis that cobalt acts through a nonmutagenic mode of action (Bucher et al., 1999; NTP, 1998).

Biological plausibility and coherence

Sustained cell proliferation, in response to cytotoxicity, can be a significant risk factor for cancer (Correa, 1996). Sustained cytotoxicity and regenerative cell proliferation may result in the perpetuation of mutations (spontaneous or directly or indirectly induced by the chemical), resulting in uncontrolled growth. It is also possible that continuous proliferation may increase the probability that damaged DNA will not be repaired. No data on cobalt are available to directly evaluate the relationship between cell damage and reparative proliferation and the development of bronchoalveolar tumors. We do not believe is can be assumed that reparative proliferation alone causes cancer. Tissues with naturally high rates of turnover do not necessarily have high rates of cancer, and tissue toxicity in animal studies does not invariably lead to cancer. Nevertheless, regenerative proliferation associated with persistent cytotoxicity appears to be a risk factor of consequence.

Conclusions Regarding Cancer Mode of Action

Limited evidence supports both mutagenic and nonmutagenic modes of action for cobalt tumorigenicity. *In vitro* and *in vivo* studies provide evidence that cobalt is capable of eliciting genotoxic effects in mammalian cells; however, two key uncertainties remain:

(1) no direct evidence linking mutagenesis to the development of cancer is available and (2) the mutagenic potential of cobalt in respiratory cells has not been evaluated.

Results of the 3-month and 2-year inhalation studies in rats and mice (Bucher et al., 1990, 1999; NTP, 1991, 1998) are consistent with the hypothesis that cobalt acts through a nonmutagenic mode of action, based on the observations that cytotoxicity and reparative proliferation occur following subchronic exposure and bronchoalveolar tumors develop at exposure levels that produce cytotoxicity and reparative proliferation. However, NTP (19998) study did not establish a clear threshold for tumor development, which is more consistent with a genotoxic mechanism. Because the mutagenic mode of action is plausible, but cannot be clearly established for carcinogenicity of inhaled cobalt, we recommend that no age-adjustment of unit risk be applied to account for possible age-dependence of carcinogenic potency as described in U.S. EPA (2005b).

Quantitative Estimates of Carcinogenic Risk

Oral Exposure

We could not locate any human or animal studies examining the carcinogenicity of cobalt following oral exposure. Therefore, derivation of an oral slope factor is precluded.

Inhalation Exposure

As available human studies were not sufficiently detailed, particularly with regards to analysis of exposure, we chose the NTP (1998; Bucher et al., 1999) 2-year carcinogenicity study in rats and mice as the key study for the derivation of an inhalation unit risk, based on the dose-response relationship for alveolar/bronchiolar (A/B) neoplasms (adenoma and carcinoma). We adjusted the exposure concentrations in these studies to continuous exposure as follows:

$$Conc_{[ADJ]} = Conc \times \frac{5\ days/\ week}{7\ days/\ week} \times \frac{6\ hours/\ day}{24\ hours/\ day}$$

This adjustment resulted in duration-adjusted concentrations of 0, 0.012, 0.040 and 0.120 mg cobalt/m^3, respectively, for the control groups: 0.3, 1 and 3 mg /m^3 (as cobalt sulfate hexahydrate). Using the RDDR computer program, as specified in the RfC guidelines (U.S. EPA, 1994b), we calculated human equivalent concentrations (HECs, in mg cobalt/m^3) at each exposure level for each species and sex using body weight default values (U.S. EPA, 1994b), assuming exposure to particulates (MMAD=1.5 μm, σ_g=2.2) with effects occurring in the thoracic region of the respiratory tract. Table 5 shows the resulting HECs.

Table 5. Human Equivalent Concentrations Corresponding to Exposure Concentrations in the NTP (1998; Bucher et al., 1999) Chronic Cancer Bioassay

Study	Male Rat	Female Rat	Male Mouse	Female Mouse
RDDR Multiplier	0.83	0.79	1.48	1.44
Control	0	0	0	0
Low	0.010	0.0095	0.018	0.017
Medium	0.033	0.032	0.059	0.058
High	0.10	0.095	0.18	0.17

We fit all models for quantal data in the U.S. EPA Benchmark Dose (BMD) software (version 1.3.2) to incidence for tumors (combined A/B adenomas and carcinomas), in rats and mice; we modeled the males and females separately. All data sets modeled showed a statistical trend for increased tumor incidence with increasing exposure concentration. In accordance with the U.S. EPA (2000) BMD methodology, the default benchmark response (BMR) of 10% increase in extra risk was used as the basis for the BMD, with the BMDL represented by the 95% lower confidence limit on the BMD. We ran the models using the default restrictions on parameters built into the BMD software. Table 6 shows the exposure concentration and incidence data that we modeled.

Table 7 summarizes the BMD modeling results. We derived all BMDLs shown from acceptable model fits ($p>0.5$). As is shown in Table 7, BMDLs were similar across study groups (range: 0.011-0.035 mg/m^3). We chose lung tumors in female rats as the endpoint for use as a point of departure for derivation of the inhalation unit risk. The BMDL for this endpoint was the lowest for all study groups (i.e., male and female rats and mice) and was based on a model that showed a good fit to the data ($p=0.84$), as reflected in the proximity of the BMDL to the BMD, after dropping the high exposure group. Dropping the high exposure group is recommended according to U.S. EPA (2000) procedure when no models achieve adequate fit using all exposure levels. Although this left only two exposure levels (in addition to the control), these exposure levels are in the low-dose portion of the curve within the region of the dose-response relationship in which response is increasing with exposure level (i.e., the region of interest for deriving the point of departure) and bracket the derived BMD. Appendix A presents the results from all model runs used to support this toxicity assessment.

Table 6. Neoplasm Incidence Observed in the NTP (1998; Bucher et al., 1999) Chronic Cancer Bioassay

Animal/Strain/Site	Incidence of Neoplasms			
	Human Equivalent Concentration of Cobalt (mg/m^3)			
F-344 Rats (male)	**0**	**0.010**	**0.033**	**0.10**
Lung: A/B adenoma or carcinoma	1/50	4/50	4/48	7/50
	Human Equivalent Concentration of Cobalt (mg/m^3)			
F-344 Rats (female)	**0**	**0.0095**	**0.032**	**0.095**
Lung: A/B adenoma or carcinoma	0/50	3/49	15/50	15/50
	Human Equivalent Concentration of Cobalt (mg/m^3)			
B6C3F1 Mice (male)	**0**	**0.018**	**0.059**	**0.18**
Lung: A/B adenoma or carcinoma	11/50	14/50	19/50	28/50
	Human Equivalent Concentration of Cobalt (mg/m^3)			
B6C3F1 Mice (female)	**0**	**0.017**	**0.058**	**0.17**
Lung: A/B adenoma or carcinoma	4/50	7/50	13/50	18/50

Table 7. Summary of BMD Modeling Results for Cobalt Cancer Data

Tumor	Species	Sex	BMD (mg/m^3)	BMDL (mg/m^3)
Lung: A/B adenoma or carcinoma	rat	male	0.085	0.035
Lung: A/B adenoma or carcinoma	rat	female	0.014[a]	0.011[a]
Lung: A/B adenoma or carcinoma	mouse	male	0.026	0.015
Lung: A/B adenoma or carcinoma	mouse	female	0.038	0.023

[a] Based on control, low and middle exposure levels; high exposure level was dropped due to failure of models to achieve adequate fit using all exposure levels.

In the absence of data to firmly support a nongenotoxic mode of action for cobalt, we calculated an inhalation cancer unit risk by linear extrapolation of the BMDL to zero exposure level (U.S. EPA, 2005a). We calculated the provisional **inhalation unit risk of 9 $(mg/m3)^{-1}$** for cobalt as follows:

Provisional Unit Risk = BMR / BMDL
= 0.1 / 0.011
= **9 $(mg/m^3)^{-1}$**

Table 8 shows continuous life-time exposure concentrations that correspond with specified risk levels (i.e., $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$).

Table 8. Continuous Life-time Exposure Concentrations Corresponding to Specified Cancer Risk

Exposure Concentration at $1x10^{-4}$ Risk	$1.1x10^{-5}$ mg/m^3
Exposure Concentration at $1x10^{-5}$ risk	$1.1x10^{-6}$ mg/m^3
Exposure Concentration at $1x10^{-6}$ Risk	$1.1x10^{-7}$ mg/m^3

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APPENDIX A: SUMMARY OF BMD MODELING OF TUMOR INCIDENCE DATA IN MALE AND FEMALE RATS AND MICE (NTP, 1998; BUCHER ET AL., 1999)

Male rat – A/B adenoma or carcinoma:
All models show acceptable fit ($p > 0.1$)
Log-logistic model yielded best fit (highest p-value and lowest AIC)
Best estimate of BMDL = 0.035 mg/m^3

Model	p	AIC	BMD mg/m^3	BMDL mg/m^3
gamma (power ≥1)	0.502	111.12	0.087	0.043
logistic	0.446	111.52	0.099	0.066
log logistic (slope ≥1)	0.510	111.07	0.085	**0.035**
2 degree polynomial (pos betas)	0.502	111.12	0.087	0.043
1 degree polynomial (pos betas)	0.502	111.12	0.087	0.043
probit	0.453	111.47	0.098	0.063
log probit (slope ≥1)	0.357	112.11	0.104	0.064
quantal linear	0.502	111.12	0.087	0.043
quantal quadratic	0.373	111.99	0.010	0.069
weibull (power ≥1)	0.502	111.12	0.087	1.043

Output from BMD v1.3.2 is shown below:

Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
Input Data File: C:\PROJECTS\COBALT\BMDS\RAMALULOG.(D)
Gnuplot Plotting File: C:\PROJECTS\COBALT\BMDS\RAMALULOG.plt
Fri Sep 09 11:46:38 2005

BMDS MODEL RUN

The form of the probability function is

P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

Dependent variable = INRM
Independent variable = ECRM
Slope parameter is restricted as slope ≥ 1

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to 1e-008

Parameter Convergence has been set to 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.02
intercept = 0.683504
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point or have been specified by the user
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.63
intercept	-0.63	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0.0398603	0.0231667
intercept	0.272287	0.592931
slope	1	NA

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	p-value
Full model	-52.8567			

Fitted model	-53.5353	1.35715	2	0.5073
Reduced model	-55.5862	5.45902	3	0.1411

AIC: 111.071

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
0.0000	0.0399	1.993	1	50	-0.7178
0.0100	0.0523	2.615	4	50	0.8797
0.0330	0.0797	3.827	4	48	0.09207
0.1000	0.1513	7.565	7	50	-0.2228

Chi-square = 1.35 DF = 2 p-value = 0.5099

Benchmark Dose Computation

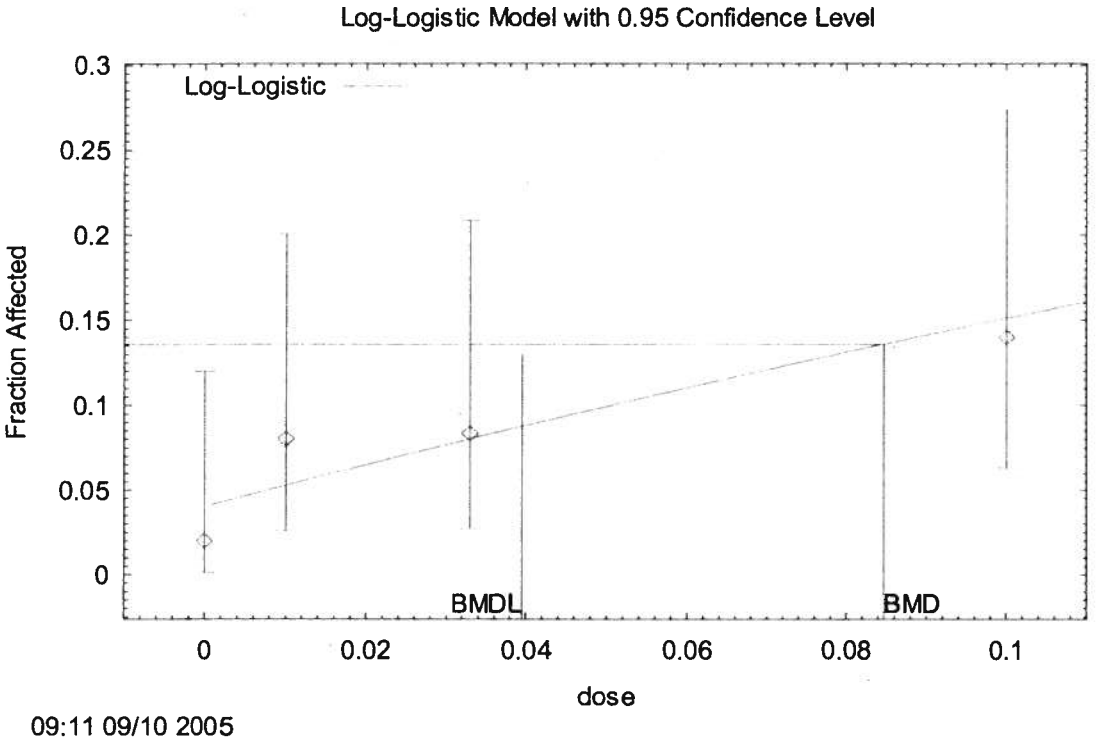
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0846262

BMDL = 0.0394914


Log-Logistic Model with 0.95 Confidence Level
Log-Logistic
Fraction Affected
0.3
0.25
0.2
0.15
0.1
0.05
0
BMDL
BMD
0
0.02
0.04
0.06
0.08
0.1
dose
09:11 09/10 2005

Female rat – A/B adenoma or carcinoma:
Most models showed poor fit ($p < 0.05$) with highest exposure level included (no increase in incidence at the highest exposure level.
The log-logistic model showed the best fit (p=0.11, lowest AIC)

Model	p	AIC	BMD mg/m^3	BMDL mg/m^3
gamma (power ≥1)	0.025	155.21	0.018	0.043
logistic	0.000	167.02	0.045	0.036
log logistic (slope ≥1)	0.090	152.86	0.015	**0.011**
2 degree polynomial (pos betas)	0.025	155.21	0.018	0.014
1 degree polynomial (pos betas)	0.025	155.21	0.018	0.014
probit	0.000	166.25	0.042	0.033
log probit (slope ≥1)	0.000	166.51	0.032	0.023
quantal linear	0.025	155.21	0.018	0.014
quantal quadratic	0.000	170.16	0.052	0.040
weibull (power ≥1)	0.025	155.21	0.018	0.014

Omitting the data from the highest exposure level improved fit of all models ($p > 0.1$)
Log-probit model yielded best fit (highest p-value and lowest AIC)
Best estimate of BMDL=0.011 mg/m^3

Model	p	AIC	BMD mg/m^3	BMDL mg/m^3
gamma (power ≥1)	1.000	87.66	0.013	0.0077
logistic	0.242	89.69	0.020	0.0164
log logistic (slope ≥1)	1.000	87.66	0.013	0.0071
2 degree polynomial (pos betas)	0.710	87.66	0.014	0.0077
1 degree polynomial (pos betas)	1.000	86.40	0.011	0.0073
probit	0.289	89.31	0.019	0.0152
log probit (slope ≥1)	0.843	85.98	0.014	**0.0110**
quantal linear	0.710	86.40	0.011	0.0073
quantal quadratic	0.535	86.70	0.017	0.0139
weibull (power ≥1)	1.000	87.66	0.014	0.0077

Output from BMD v1.3.2 (all data included) is shown below:

==========

Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
Input Data File: C:\PROJECTS\COBALT\BMDS\RAFELU\RAFELULOGLOG.(D)

Gnuplot Plotting File:
C:\\PROJECTS\COBALT\BMDS\RAFELU\RAFELULOGLOG.plt
Fri Sep 09 16:34:38 2005

BMDS MODEL RUN

The form of the probability function is

P[response] = background + (1-background)/[1+EXP(-intercept-slope*Log(dose))]

Dependent variable = INRF
Independent variable = ECRF
Slope parameter is restricted as slope $\geq$ 1

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to 1e-008
Parameter Convergence has been set to 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = 1.93572
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept
intercept	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0	NA
intercept	1.98253	0.20995
slope	1	NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-72.3723			
Fitted model	-75.4299	6.1152	3	0.1061
Reduced model	-89.3929	34.0413	3	<.0001

AIC: 152.86

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
0.0000	0.0000	0.000	0	50	0
0.0095	0.0645	3.162	3	49	-0.09415
0.0320	0.1885	9.427	15	50	2.015
0.0950	0.4082	20.411	15	50	-1.557

Chi-square = 6.49 DF = 3 p-value = 0.0900

Benchmark Dose Computation

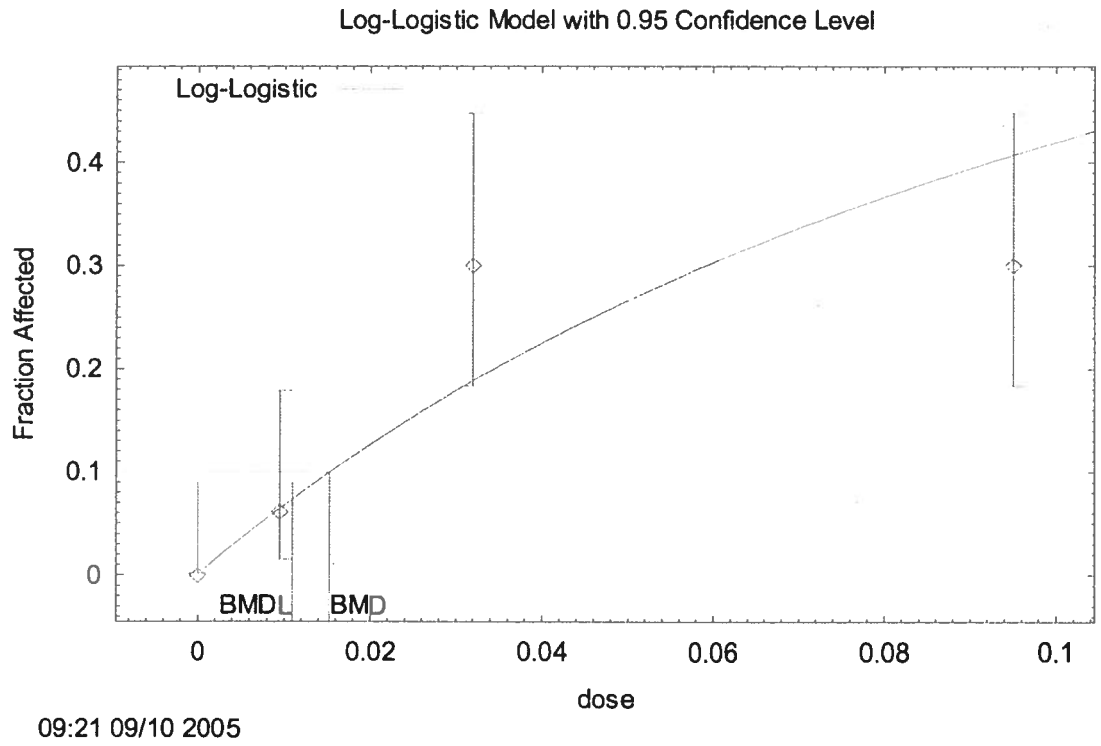
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0153022

BMDL = 0.0109172



Output from BMD v1.3.2 (highest exposure level excluded) is shown below:

```
================================================================================
        Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
        Input Data File:
C:\PROJECTS\COBALT\BMDS\RAFELUSE\RAFELUSEPROLOG.(D)
        Gnuplot Plotting File:
C:\PROJECTS\COBALT\BMDS\RAFELUSE\RAFELUSEPROLOG.plt
                                        Fri Sep 09 16:41:28 2005
================================================================================

BMDS MODEL RUN
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```

The form of the probability function is:

P[response] = Background
+ (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

where CumNorm(.) is the cumulative normal distribution function

Dependent variable = INRF
Independent variable = ECRF
Slope parameter is restricted as slope $\geq$ 1

Total number of observations = 4
Total number of records with missing values = 1
Maximum number of iterations = 250
Relative Function Convergence has been set to 1e-008
Parameter Convergence has been set to 1e-008

User has chosen the log transformed model

Default Initial (and Specified) Parameter Values
background = 0
intercept = 3.0285
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept
intercept	1

Parameter Estimates

Variable	Estimate	Std. Err.

background	0	NA
intercept	2.97347	0.157916
slope	1	NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-41.8291			
Fitted model	-41.9887	0.319256	2	0.8525
Reduced model	-54.9105	26.1628	2	<.0001

AIC: 85.9774

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0000	0.000	0	50	0
0.0095	0.0462	2.263	3	49	0.5015
0.0320	0.3197	15.985	15	50	-0.2987

Chi-square = 0.34 DF = 2 p-value = 0.8434

Benchmark Dose Computation

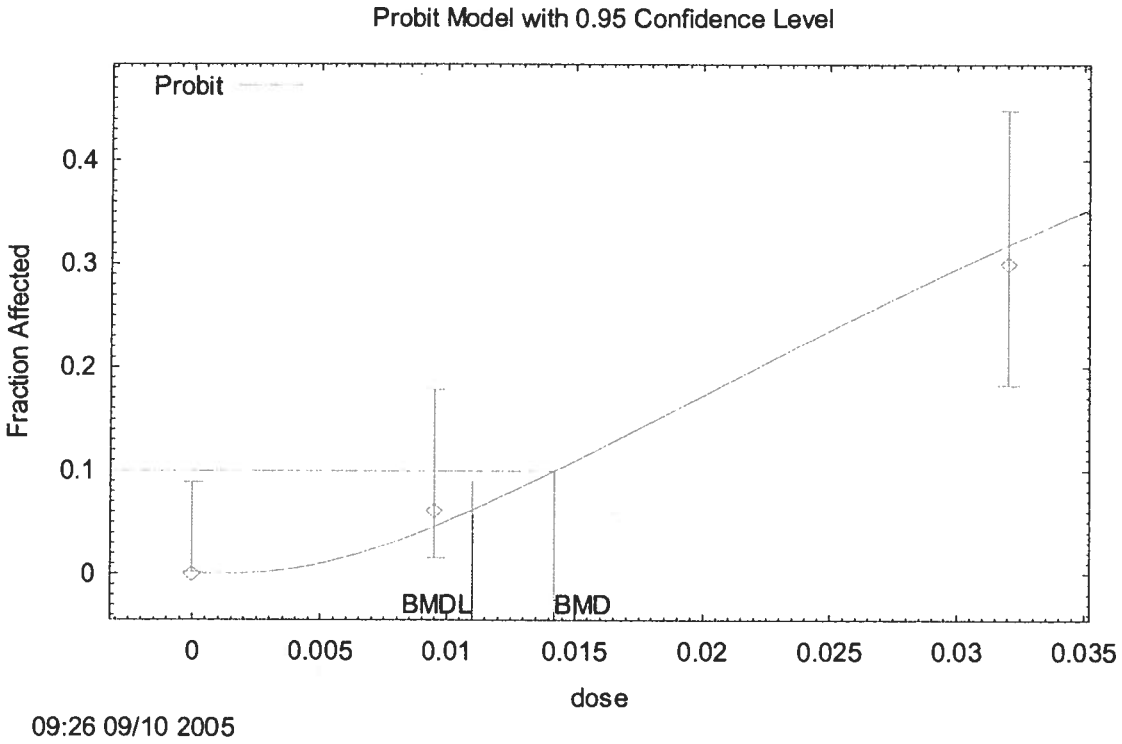
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0141927

BMDL = 0.0109984


Probit Model with 0.95 Confidence Level
Probit
Fraction Affected
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
0.005
0.01
0.015
0.02
0.025
0.03
0.035
dose
09:26 09/10 2005

Male mouse – A/B adenoma or carcinoma:
All models show acceptable fit ($p > 0.1$)
Log-logistic model yielded best fit (highest p-value and lowest AIC)
Best estimate of BMDL = 0.015 mg/m^3

Model	p	AIC	BMD mg/m^3	BMDL mg/m^3
gamma (power ≥1)	0.944	251.10	0.033	0.0215
logistic	0.759	251.54	0.048	0.0359
log logistic (slope ≥1)	0.999	250.99	0.026	**0.0150**
2 degree polynomial (pos betas)	0.944	251.10	0.033	0.0215
1 degree polynomial (pos betas)	0.944	251.10	0.033	0.0215
probit	0.775	251.50	0.046	0.0349
log probit (slope ≥1)	0.594	252.03	0.059	0.0397
quantal linear	0.944	251.10	0.033	0.0215
quantal quadratic	0.412	252.76	0.080	0.0633
weibull (power ≥1)	0.944	251.10	0.033	0.0215

Output from BMD v1.3.2 is shown below:

```
================================================================================
        Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
        Input Data File: C:\ROJECTS\COBALT\BMDS\MOMALU\MOMALULOGLOG.(D)
        Gnuplot Plotting File:
C:\PROJECTS\COBALT\BMDS\MOMALU\MOMALULOGLOG.plt
                                        Fri Sep 09 16:57:38 2005
================================================================================

BMDS MODEL RUN
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  The form of the probability function is

  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


  Dependent variable = INMM
  Independent variable = ECMM
  Slope parameter is restricted as slope ≥ 1

  Total number of observations = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
```

Relative Function Convergence has been set to 1e-008
Parameter Convergence has been set to 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.22
intercept = 1.47367
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.62
intercept	-0.62	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0.22179	0.0478621
intercept	1.45848	0.375385
slope	1	NA

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	p-value

Full model	-123.493			
Fitted model	-123.494	0.00271986	2	0.9986
Reduced model	-130.684	14.3818	3	0.002429

AIC: 250.988

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
0.0000	0.2218	11.090	11	50	-0.03047
0.0180	0.2777	13.884	14	50	0.03649
0.0590	0.3793	18.963	19	50	0.0109
0.1800	0.5613	28.065	28	50	-0.0185

Chi-square = 0.00 DF = 2 p-value = 0.9986

Benchmark Dose Computation

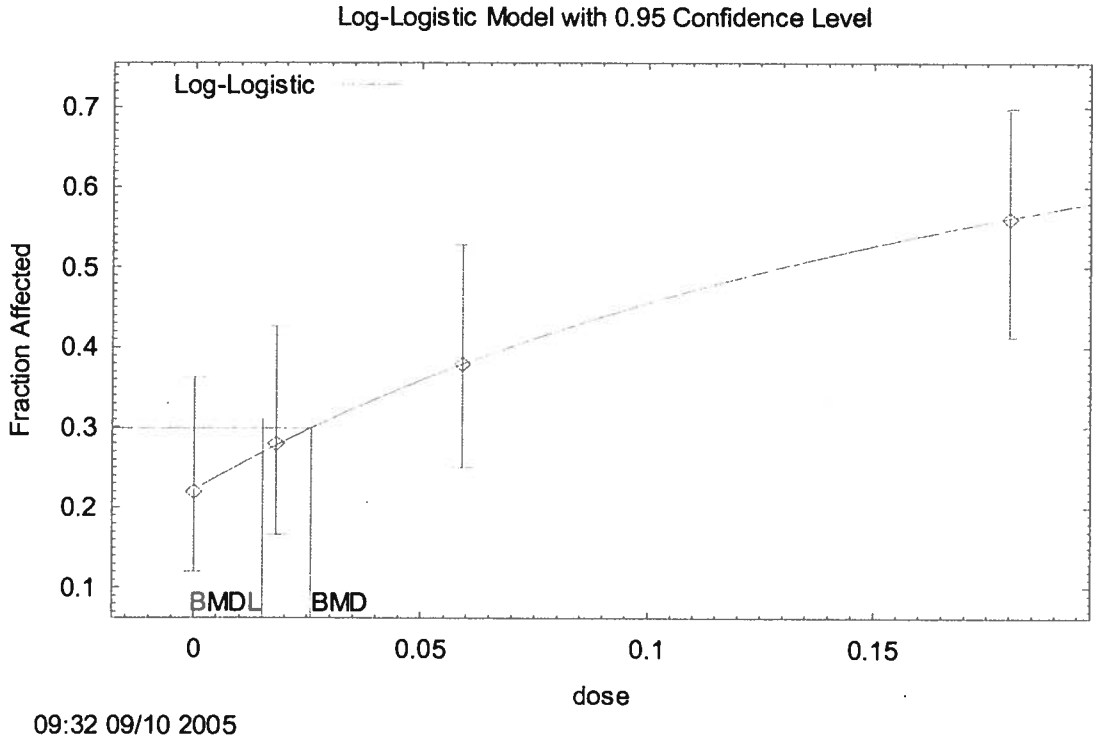
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0258434

BMDL = 0.0149697


Log-Logistic Model with 0.95 Confidence Level
Log-Logistic
Fraction Affected
0.7
0.6
0.5
0.4
0.3
0.2
0.1
BMDL
BMD
0
0.05
0.1
0.15
dose
09:32 09/10 2005

Male mouse – A/B adenoma or carcinoma:
All models show acceptable fit ($p > 0.1$)
Log-logistic model yielded best fit (highest p-value and lowest AIC)
Best estimate of BMDL = 0.023 mg/m^3

Model	p	AIC	BMD mg/m^3	BMDL mg/m^3
gamma (power ≥1)	0.571	196.12	0.0455	0.0296
logistic	0.273	197.61	0.0735	0.0562
log logistic (slope ≥1)	0.700	195.72	0.0384	**0.0231**
2 degree polynomial (pos betas)	0.571	196.12	0.0455	0.0296
1 degree polynomial (pos betas)	0.571	196.12	0.0455	0.0296
probit	0.300	197.42	0.0697	0.0528
log probit (slope ≥1)	0.167	198.57	0.0768	0.0524
quantal linear	0.571	196.12	0.0455	0.0296
quantal quadratic	0.117	199.26	0.0959	0.0739
weibull (power ≥1)	0.571	196.12	0.0455	0.0296

Output from BMD v1.3.2 is shown below:

```
==========================================================================
        Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
        Input Data File: C:\PROJECTS\COBALT\BMDS\MOFELU\MOFELULOGLOG.(D)
        Gnuplot Plotting File:
C:\PROJECTS\COBALT\BMDS\MOFELU\MOFELULOGLOG.plt
                                        Fri Sep 09 17:05:08 2005
==========================================================================

BMDS MODEL RUN
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  The form of the probability function is

  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


  Dependent variable = INMF
  Independent variable = ECMF
  Slope parameter is restricted as slope ≥ 1

  Total number of observations = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to 1e-008
```

Parameter Convergence has been set to 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.08
intercept = 1.17812
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.6
intercept	-0.6	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0.0920048	0.035283
intercept	1.06119	0.354864
slope	1	NA

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	p-value
Full model	-95.5104			

Fitted model	-95.8619	0.702985	2	0.7036
Reduced model	-102.791	14.5619	3	0.002232

AIC: 195.724

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
0.0000	0.0920	4.600	4	50	-0.2937
0.0170	0.1345	6.726	7	50	0.1135
0.0580	0.2223	11.117	13	50	0.6403
0.1700	0.3911	19.556	18	50	-0.451

Chi-square = 0.71 DF = 2 p-value = 0.7003

Benchmark Dose Computation

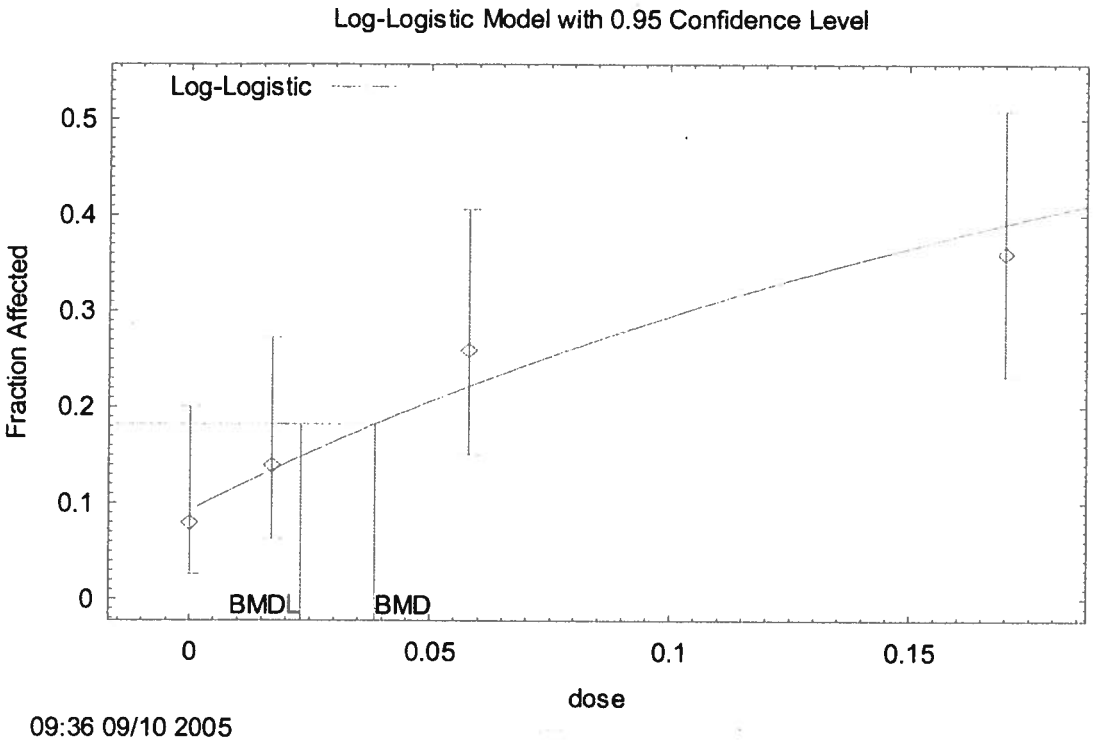
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0384492

BMDL = 0.0231


Log-Logistic Model with 0.95 Confidence Level
Log-Logistic
Fraction Affected
0.5
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
0.05
0.1
0.15
dose
09:36 09/10 2005

APPENDIX B: COBALT DAILY INTAKE VALUES

Dabeka and McKenzie, 1995
H&W Canada market basket sample from Montreal Canada (1988)

	Cobalt Intake		
	MF	M	F
Age	ug/kg/d	ug/kg/d	ug/kg/d
1-4 yr	0.49	ND	ND
5-11 yr	0.34	ND	ND
12-19 yr	ND	0.23	0.18
20-39 yr	ND	0.20	0.14
40-65 yr	ND	0.15	0.13
>65 yr	ND	0.13	0.12
All	0.13	ND	ND

Pennington and Jones 1987
FDA market basket (Total Diet Survey), 1984

	Cobalt Intake		
	MF	M	F
Age	ug/kg/d	ug/kg/d	ug/kg/d
6-11 mo	0.37	ND	ND
2 yr	0.39	ND	ND
14-16 yr	ND	0.19	0.14
25-30 yr	ND	0.14	0.11
60-65 yr	ND	0.14	0.09

M: Males, F: Females, MF: Male and Females